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# THE BOTANICAL GAZETTE

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EDITOR  
E J KRAUS

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VOLUME 99

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WITH TWO PLATES AND SIX HUNDRED AND EIGHTY-FOUR FIGURES



THE UNIVERSITY OF CHICAGO PRESS  
CHICAGO, ILLINOIS

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THE CAMBRIDGE UNIVERSITY PRESS, LONDON  
THE MARUZEN COMPANY, LIMITED, TOKYO  
THE COMMERCIAL PRESS, LIMITED, SHANGHAI

PUBLISHED  
SEPTEMBER, DECEMBER, 1937, AND MARCH, JUNE, 1938

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COMPOSED AND PRINTED BY THE UNIVERSITY OF CHICAGO  
PRESS, CHICAGO, ILLINOIS, U.S.A.

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# THE BOTANICAL GAZETTE

*September 1937*

## SOME CHROMOSOME COMPLEMENTS IN THE CACTACEAE AND A STUDY OF MEIOSIS IN *ECHINOCEREUS PAPILLOSUS*<sup>1</sup>

ELEANOR COOKE BEARD

(WITH PLATES I, II AND TEXT FIGURES)

### Introduction

The cytology of the Cactaceae is of particular interest because of the development in the family of certain morphological characters which sharply separate it from all others. As a whole, this family contains well over 1000 species in 124 genera. The material for this paper was taken entirely from the tribe Cereeae. Since the evidence indicates that this is the most highly evolved section, it seems reasonable to suppose that as wide a range of variation in chromosome numbers will be found here as in either of the other two tribes. This paper reports briefly the results of a cytological study of forty-six species, including two forms and one species thought to be a hybrid. The chromosome complements of these species are described and their relationships to the taxonomy and phylogeny of the group are discussed. It is hoped that this contribution, supplemented by future work, may determine whether there are cytological peculiarities associated with taxonomic relationships already established on the basis of morphological characters. More particularly, series of polyploid and aneuploid chromosome numbers may be built up which may establish possible lines of evolution and points at which new species have arisen.

<sup>1</sup> Papers from the Department of Botany, University of Michigan, no. 614.



Since there was no account of meiosis in the Cactaceae, a careful study was made of microsporogenesis in *Echinocereus papillosus* Linke, a representative of one of the more complex genera.

TISCHLER (28) lists two counts of chromosomes in the *Neomammillaria*, one by JARETZKY (16) and the other by ISHII (14), both giving the haploid number as eleven. SUGIURA (25), in his list of chromosome numbers in the angiosperms, reports the diploid number of twenty-four in two species of *Neomammillaria* and one of *Zygocactus* (*Epiphyllum*). *Opuntia brasiliensis* is reported by JOHANSEN (17) as having a diploid number of twenty-two. STOCKWELL (24) gives haploid numbers of nine, eleven, twenty-two, and thirty-three from seventeen types. The brief paper by JOHANSEN and the longer one by STOCKWELL, both concerned with chromosomes as found in root tips, offer the first substantial contribution to the knowledge of the chromosomes of this group.

### Material and methods

The material for this study was derived from a number of sources. Part of it has been under cultivation for some time at the University of Michigan Botanical Garden and came originally from the collections of the Missouri Botanical Garden. Most of the Central American species, with those from Tamaulipas, Mexico, were collected by Prof. H. H. BARTLETT. Those from the San Luis Potosi area were obtained by Dr. CYRUS LUNDELL. The remainder were collected by Dr. ELZADA CLOVER and the writer in the lower Rio Grande Valley, Texas.

Material was determined throughout from the monograph on the Cactaceae by BRITTON and ROSE (3).

The species which bloomed freely provided ample material for the study of the chromosomes of pollen mother cells during microsporogenesis. Counts were obtained in other species from root tips, particularly from certain species with characteristic aerial roots.

*Neomammillaria* and related genera were difficult to handle because the buds did not emerge at the bases of the papillae until almost mature. It was necessary to remove them as soon as the wool in which they were imbedded began to push out. At this time they

could be lifted out by inserting a small scalpel at the base of the papilla and prying gently.

To determine the stage of meiosis in buds, anthers were examined in aceto-carmine smears. It was found that meiotic divisions were most numerous from 10 A.M. until 2 P.M. Diakinesis stages were most frequently found just before noon. To obtain a successful smear, as many as ten anthers were required because various stages were often represented in the same bud.

Buds in good condition were split. One half was stripped of sepals, fixed at once in Navashin's fluid (Karpechenko formula), dehydrated through a long series of alcohols, and later imbedded. The other half was used to make permanent smears, after the method of TAYLOR (26). For smear preparations fixation in Navashin's fluid also proved most satisfactory.

The schedule used in staining smears was essentially SMITH's (23) modification of the Gram stain. However, crystal violet in 0.5 of 1 per cent solution and the iodine-potassium-iodide similarly diluted proved easier to control. The picric acid wash was a saturated solution diluted four times. This method of staining gave a clear yellow cytoplasm and purple chromosomes.

Root tips were fixed in Allen's modification of Bouin's fluid, Zenker's fluid, modified Gilson's fluid, chrom-acetic, Navashin's fluid, and strong Flemming diluted about one-half. The last was most efficient. Mitoses went on rapidly from 10 A.M. until early afternoon. Tips from aerial roots were cut from the plant and allowed to stand for a few minutes in distilled water. This softened the mucilage that covered the root so that it could be largely removed by wiping with cotton. When the root tips were no longer slippery, they were slit up one side to aid penetration and cut back to within 5 mm. of the tip.

Iron-alum haematoxylin proved to be a more valuable stain for root tips than crystal violet, particularly when picric acid was used as a destaining agent (29).

### **Microsporogenesis in *Echinocereus papillosus***

*Echinocereus papillosus* Linke, selected for a study of meiosis, is native to southern Texas and is found over a large area. Both sec-

tioned and smeared material were used. Prophase smears were found to be of little value. The chromosomes in the Cactaceae are minute and not favorable for critical studies on chromosome structure.

The resting nucleus of the microsporocyte is less than  $10\mu$  in thickness (fig. 1)<sup>2</sup> and presents the reticulum of fine threads characteristic of this stage. There is usually only one nucleolus. This body stains deeply during early prophase and does not begin to lose its chromaticity until late pachynema. It does not disappear until just before metaphase I, when it fades gradually but does not change from its spherical form.

With the beginning of prophase, the stage of leptonema (fig. 2) becomes clear. There is a definite thickening of the threads, and at the same time they show a tendency to run parallel to one another, which is sharply defined just before the stage of synizesis (fig. 3). At this time the nucleus begins to increase in size, and with this enlargement is possibly associated the condition of apparent contraction of the chromatic material, which may be due at least in part to imperfect fixation. The late stages of leptonema show a definite pairing of threads, which become closely associated. As has been pointed out by others, this association of threads (fig. 4) in a parasynaptic relation does not take place simultaneously all along the threads, but begins at random points.

With the increase in size of the nucleus, a marked thickening of the threads takes place which carries the nucleus into pachynema (figs. 5, 6). The parallel threads now become closely associated and apparently twisted. This is accompanied by a marked shortening of the elements. Coincident with this stage is the appearance of free ends at the periphery of the nucleus (fig. 7). In this species the tetrad structure of the chromosome is not obvious at metaphase of the first division. The lengthwise splitting of each chromosome may take place much earlier, but it is not apparent until early anaphase of the first division.

The close approximation of the synaptic mates continues through late pachynema, with further condensation of the threads (fig. 8). Finally during early diakinesis an apparent loosening of the association occurs which frees the members of the pairs except at one or

<sup>2</sup> See plate I, opposite page 20.

both ends (figs. 9, 10). The pairs of chromosomes condense still further and assume the forms characteristic of diakinesis (fig. 11). At this time the nucleus begins to decrease in size until it is again approximately  $10\mu$  in thickness (fig. 12).

The chromosomes as they appear at metaphase I (fig. 13) are oval or round in outline, with an average width of from  $1.7$  to  $2.0\mu$  and a length of from  $2.0$  to  $2.5\mu$ . These dimensions represent an average for all the species studied. Throughout the tribe there is little difference in chromosome size at meiosis. The spindle establishes itself quickly following diakinesis, and the chromosomes become arranged at the plate. As seen from the pole, they are usually placed in a circle of eight with three in the center. The spindle fiber attachment for most of the chromosomes is median, but in some it is terminal. The chromosomes at anaphase I barely reach the poles before they show that they are split (fig. 14).

During interkinesis the sister chromatids tend to separate and to elongate (fig. 15), but again condense in preparation for the second division. The nucleolus which appeared at the beginning of interkinesis fades away soon after the disappearance of the nuclear membrane (fig. 16). Metaphase II is shown in figure 17 and anaphase in figure 18. The four daughter nuclei, after a long telophase, return to a resting condition (fig. 19). Furrows develop at the periphery of the sporocyte and proceeding inward apparently cut out the microspores (fig. 20).

In this species the pollen is almost wholly fertile, and there is no evidence of meiotic irregularity. The absence of lagging and non-disjunction indicates that the material is not of hybrid origin.

A matter of great interest was the appearance in this species, as well as in the majority of others studied, of certain bodies in the cytoplasm whose function and origin are obscure. They were first noticed in the material during interkinesis. They may be seen in early prophase and through diakinesis as two spherical or oval bodies at opposite ends of the cell (fig. 5). During metaphase I they arrange themselves on either side of the spindle. At anaphase I and through interkinesis and prophase II they lie close to the periphery of the cell, where they are conspicuous. At any time after metaphase I, four such bodies may be present. During interkinesis, two of the four

bodies are generally much more prominent (fig. 15) and remain so through prophase II (fig. 16). As the nuclei pass into metaphase II, the bodies become much less evident but are visible again in second anaphase (figs. 17, 18). When telophase II is initiated the four bodies come to lie one at the side of each nucleus, and shortly there are two by each nucleus. They fade away at this point.

The only reference to such structures noted in the literature is that of CASTETTER (5) in his paper on *Melilotus*. He figures similar bodies on either side of the metaphase I plate. In his discussion he refers to them as centrosome-like bodies, but states that he is unable to come to any conclusion as to their history or function.

The only further information which this study can offer is the reaction which these structures give to various stains and reagents. In general their staining reactions follow that of the chromatic material. It is necessary that the stain be left rather heavily in the nucleus, if these bodies are to show clearly. In prophase, when the nucleolus was prominent, they were more obscure. They are sharpest just before the formation of the metaphase II plate.

The bodies are not destroyed by fluids containing chromic acid, formaldehyde, or acetic acid. They also occur when material is fixed in a modification of Bouin's fluid in which anthroquinone replaces picric acid (1). They are partially or completely destroyed by a diluted Gilson's fluid. They show fairly well in sections or smears when stained with iron-alum haematoxylin. Crystal violet and safranin both bring them out brilliantly; the best results in the use of both stains are obtained when a counter wash of picric acid is employed. Alizarin will also stain them, but not sharply.

### Chromosome configurations in other species

The results of the examinations of other species are given in the order in which the species are arranged by BRITTON and ROSE. The stages of meiosis best adapted for studies of chromosome configuration are diakinesis and late interkinesis. In root tips the metaphase plate is most useful.

The chromosomes in meiosis are compact and so small that peculiarities of size and form are difficult to recognize. Usually one pair is larger than the others, two to four pairs are medium sized,

and the remaining pairs consist of very small and almost spherical chromosomes.

Drawings of the chromosomes in the root tips were made from cells near the outermost layer, since these cells are larger and likely to be better fixed than those in the interior. They were never taken from further in than the sixth row and usually from the second or third.

In the following list, the numbers placed after the species names are accession numbers at the Botanical Garden of the University of Michigan.

1. *Wilcoxia poselgeri* (Lemaire) Br. & R. (15275): Diploid, twenty-two chromosomes; eleven pairs at metaphase I of meiosis (fig. 21); chromosomes all about the same size, ten pairs with median spindle fiber attachment, one with terminal attachment.

2. *Nyctocereus serpentinus* (Lagasca & Rod.) Br. & R. (13918): Diploid, twenty-two chromosomes; eleven pairs shown at metaphase I of meiosis (fig. 22); spindle fiber attachments seem to be terminal or slightly subterminal.

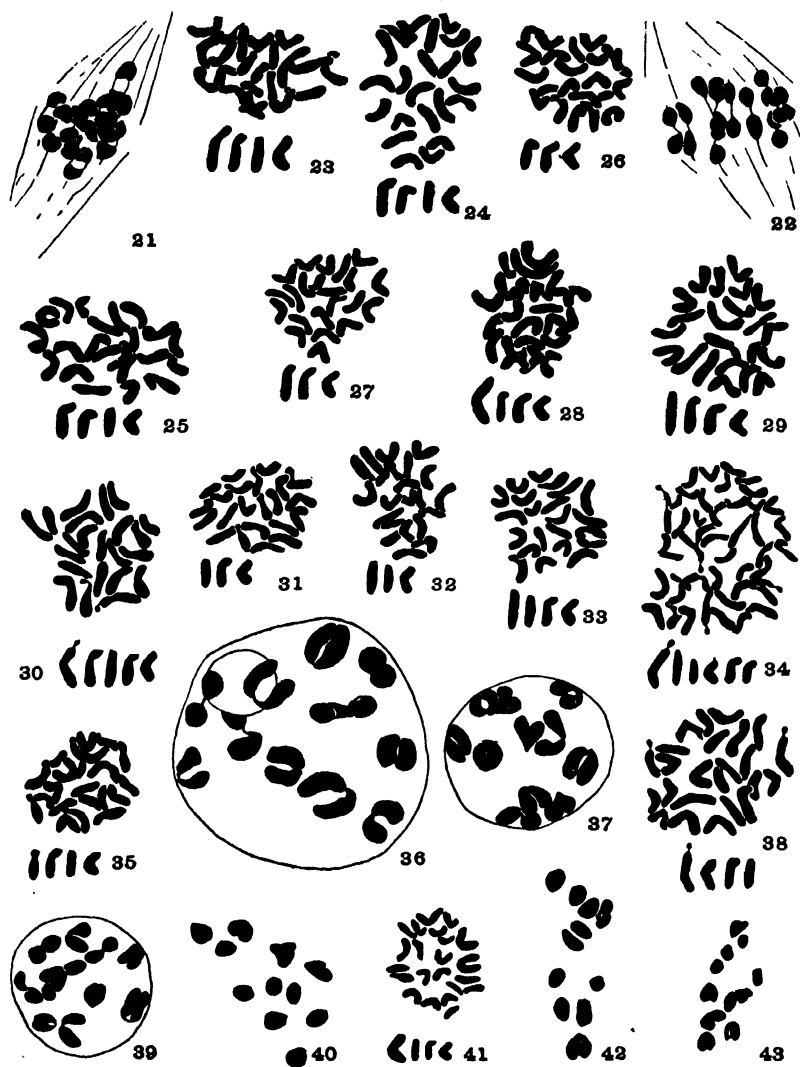
3. *Acanthocereus pentagonus* (Linnaeus) Br. & R. (15277): Diploid, twenty-two chromosomes; root tip metaphase (fig. 23); two chromosomes large with submedian attachments, four subterminal, six terminal, ten median or nearly median.

4. *Hylocereus guatemalensis* (Eichlam) Br. & R. (13941): Diploid, twenty-two; four groups of chromosomes in root tip metaphase (fig. 24); two largest chromosomes have spindle fiber attachments subterminal, two submedian, six terminal, twelve median varying considerably in size.

5. *Hylocereus purpusii* (Weingart) Br. & R. (13900): Diploid, twenty-two; root tip metaphase (fig. 25); chromosomes fall into the same groups as do those of the preceding species, to which it is closely related.

6. *Hylocereus undatus* (Haworth) Br. & R. (1526): Diploid, twenty-two; root tip metaphase (fig. 26); four chromosomes with subterminal attachments, six with submedian, twelve median; chromosomes more uniform in size and shape than those in the preceding two species.

7. *Hylocereus cubensis* Br. & R. (13997): Diploid, twenty-two; root tip metaphase (fig. 27); since this species is closely related to



FIGS. 21-43.—Fig. 21, *Wilcoxia poselgeri*, metaphase I. Fig. 22, *Nyctocereus serpen-  
tinus*, metaphase I. Root tip metaphase of: fig. 23, *Acanthocereus pentagonus*; fig. 24,  
*Hylocereus guatemalensis*; fig. 25, *Hylocereus purpusii*; fig. 26, *H. undatus*; fig. 27,  
*H. cubensis*; fig. 28, *H. monacanthus*; fig. 29, *H. triangularis*; fig. 30, *Selenicereus hon-  
durensis*; fig. 31, *S. pteranthus*; fig. 32, *S. kunthianus*; fig. 33, *S. spinulosus*; fig. 34,  
*Mediocactus coccineus* (with 44 chromosomes); fig. 35, *Werkleocereus glaber*. Fig. 36,  
*Echinocereus angusticeps*, diakinesis. Fig. 37, *E. pentalophus*, diakinesis. Fig. 38, *E.  
blanchii*, root tip metaphase, diploid 24 chromosomes. Fig. 39, *E. reichenbachii*, diakine-  
sis. Fig. 40, *E. fitchii*, prophase II. Fig. 41, *Lophophora williamsii*, root tip metaphase.  
Fig. 42, *Hamatocactus setispinus* var. *hamatus*, prophase II. Fig. 43, *H. setispinus* var.  
*setaceus*, prophase II.

*H. undatus*, it would be expected to have similar chromosome complements, which is the case.

8. *Hylocereus monacanthus* (Lemaire) Br. & R. (14067): Diploid, twenty-two; root tip metaphase (fig. 28) shows chromosomes of this species distinctly different from others in the genus; two chromosomes half again as long as the next largest appear to have median attachments, two subterminal, eight submedian, ten median.

9. *Hylocereus triangularis* (Linnaeus) Br. & R. (1569): Diploid, twenty-two; root tip metaphase (fig. 29); two chromosomes longer than the rest, with terminal attachments, four subterminal, six submedian, ten median.

10. *Selenicereus hondurensis* (Schumann) Br. & R. (14688): Diploid, twenty-two; root tip metaphase (fig. 30); two chromosomes of the set with spindle fiber attachments submedian have satellites on the shorter arm, four have attachments subterminal, ten terminal, four submedian, two median.

11. *Selenicereus pteranthus* (Linke & Otto) Br. & R. (1547): Diploid, twenty-two; root tip metaphase (fig. 31) presents chromosomes similar in size and form; six short with terminal attachments, six subterminal, ten median.

12. *Selenicereus kunthianus* (Otto) Br. & R. (1360): Diploid, twenty-two; root tip metaphase (fig. 32); eight chromosomes with terminal attachments, remainder with median or near median attachments and similar in size.

13. *Selenicereus spinulosus* (DeCandolle) Br. & R. (13697): Diploid, twenty-two; root tip metaphase (fig. 33); six chromosomes terminally attached, four have subterminal attachments, remaining twelve attachments are all nearly median.

14. *Mediocactus coccineus* (Salm-Dyck) Br. & R. (7520): Diploid, forty-four chromosomes; root tip metaphase (fig. 34); four chromosomes, among the largest in the complement, have subterminal attachments and appear to bear satellites on the longer arm, four other long chromosomes with terminal attachments, four short chromosomes with terminal attachments, sixteen with median attachments, eight subterminal and eight submedian. This species is the only tetraploid so far known in this subtribe. The material was obtained from a plant in the greenhouses of the Botanical Garden of the Uni-



versity. It has not flowered since the writer began work on the material, so there has been no opportunity for studies of meiosis. The cells of the root are noticeably larger than those of related diploid species, but the chromosomes are smaller and more slender than those found in normal diploid cells.

15. *Werkleocereus glaber* (Eichlam) Br. & R. (7553): Diploid, twenty-two; root tip metaphase (fig. 35); two chromosomes with subterminal attachments, two with attachments more markedly subterminal, two with terminal attachments, sixteen median or nearly so.

16. *Echinocereus papillosus* Linke (15255): Diploid, twenty-two; diakinesis figure shows eleven pairs of chromosomes (fig. 11).

17. *Echinocereus angusticeps* Clover (15261): Diploid, twenty-two; eleven pairs are evident at diakinesis (fig. 36); in about 20 per cent of the cells examined there is non-disjunction at metaphase I, ten and twelve chromosomes passing to either pole. This species is most closely allied to *E. papillosus* and is possibly derived from it. Morphologically it resembles that species but is much smaller. Its habitat is different from that of *E. papillosus* in that it grows in sandy loam in open woods, while the other species is found on gravel or limestone hills. The two species are found within a few miles of each other, but their ranges apparently do not overlap.

18. *Echinocereus pentalophus* (DeCandolle) Rümpler (15253): Diploid, twenty-two; eleven pairs evident at diakinesis (fig. 37); this species differs from the two in the same genus previously discussed in being a slender procumbent plant whereas the others are erect. The spines are borne on ridges rather than on papillae, and the flowers are rich mallow purple with white centers as compared with the pale yellow and maroon red of the foregoing two species.

19. *Echinocereus blanckii* (Poselger) Palmer (15268): Diploid, twenty-four; root tip metaphase (fig. 38); two chromosomes having the spindle fiber attachments subterminal bear satellites on the longer arm, twelve chromosomes have median attachments, four subterminal, six terminal. This species is morphologically different from the preceding species in the increase in the number of ribs, the presence of definite papillae bearing the spine areoles, and the appearance of flowers which are entirely mallow purple. Cytologically this spe-

cies is interesting because the haploid number of chromosomes is twelve. In cultivation the plants bloom poorly, and a large percentage of the buds abort. Such buds as mature do not show complete pollen fertility. No fruit has been reported for this species by BRITTON and ROSE. All evidence yet available suggests that *E. blanchii* came out of *E. pentalophus* by the addition of an extra chromosome through non-disjunction.

20. *Echinocereus reichenbachii* (Terschek) Haage Jr. (15262): Diploid, twenty-two; diakinesis figure presents the usual eleven pairs of chromosomes (fig. 39); the plant is one of wide range and adaptability.

21. *Echinocereus fitchii* Br. & R. (15267): Diploid, twenty-two; haploid number of eleven shown at metaphase II (fig. 40).

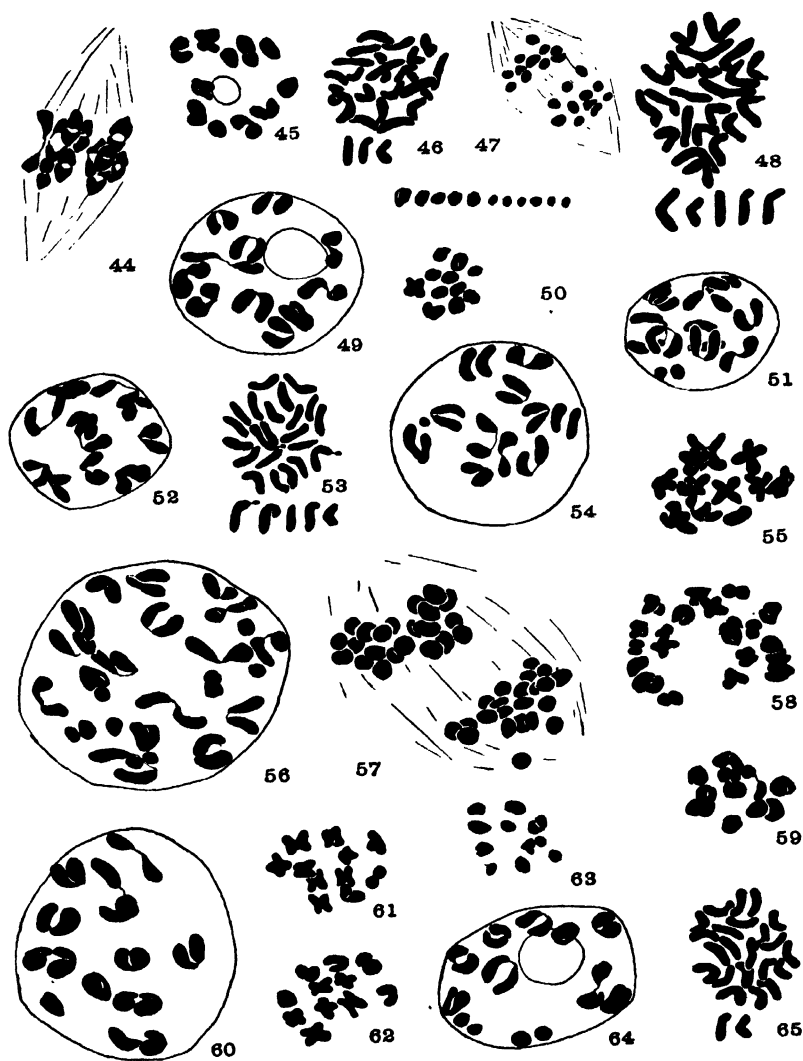
22. *Echinocereus enneacanthus* Engelm. (15256): Diploid, twenty-two (?); chromosomes were studied in root tips, all found to be nearly the same size.

23. *Lophophora williamsii* (Lemaire) Coulter (15269): Diploid, twenty-two; root tip metaphase interesting in that all but one pair of chromosomes are very small (fig. 41); two largest chromosomes have median attachments, two subterminal, four terminal, and fourteen median or nearly so.

24. *Hamatocactus setispinus* (Engelmann) Br. & R. (15295, 15297): Diploid, twenty-two; prophase II figures show eleven split chromosomes (figs. 42, 43); in the species there are two distinct forms which have been investigated. Morphological variations involving number of ribs, presence or absence of papillae on the ribs, and number and shape of the spines are found. ENGELMANN went so far as to give varietal names to the forms, but these are not recognized by BRITTON and ROSE. Observations in the field have led the writer to believe that these types should be recognized as distinct. Both have a haploid number of eleven, but there is a marked difference in chromosome size.

25. *Echinofossulocactus grandicornis* (Lemaire) Br. & R. (16142): Diploid, twenty-two (?); root tip material too scant to permit positive statement.

26. *Ferocactus hamatacanthus* (Muhlenpfordt) Br. & R. (15271):



FIGS. 44-65.—Fig. 44, *Ferocactus hamulacanthus*, metaphase I. Fig. 45, *Homaloccephala texensis*, prophase II. Fig. 46, *Astrophytum asterias*, root tip metaphase. Fig. 47, *Thelocactus bicolor*, anaphase II. Fig. 48, *Coryphantha runyonii*, diakinesis. Fig. 49, *Escobaria runyonii*, diakinesis. Fig. 50, *Dolicothele longimamma*, prophase II. Fig. 51, *Neomammillaria heyderi*, diakinesis. Fig. 52, *N. hemisphaerica*, diakinesis. Fig. 53, *N. magnimamma*, root tip metaphase. Fig. 54, *N. chionocephala*, diakinesis. Fig. 55, *N. aureiceps*, prophase II, characteristic H shaped split chromosomes. Fig. 56, *N. multiceps*, tetraploid, 44 chromosomes showing pairing. Fig. 57, *N. multiceps*, anaphase I. Fig. 58, *N. multiceps*, prophase II. Fig. 59, *N. decipiens*, prophase II. Fig. 60, *N. longicoma*, diakinesis. Fig. 61, *N. tenampensis*, prophase II. Fig. 62, *N. minuta*, prophase II. Fig. 63, *Epiphyllum strictum*, anaphase I. Fig. 64, *E. ackermannii*, diakinesis. Fig. 65, *Rhipsalis mesembrianthemoides*, root tip metaphase.

Diploid, twenty-two; figure of metaphase I shows eleven pairs of chromosomes (fig. 44).

27. *Homalocephala texensis* (Hopffer) Br. & R. (15272): Diploid, twenty-two; prophase II shows haploid number of eleven (fig. 45); this species is representative of a small genus closely related to *Echinocactus*.

28. *Astrophytum asterias* (Zuccarini) Lemaire (15274): Diploid, twenty-two; root tip metaphase (fig. 46); ten chromosomes straight with no evident constrictions, suggesting terminal attachments; two attachments subterminal, and ten with attachments median.

29. *Thelocactus bicolor* (Galeotti) Br. & R. (15294): Diploid, twenty-two; eleven chromosomes shown in anaphase II (fig. 47).

30. *Coryphantha runyonii* Br. & R. (15270): Diploid, twenty-two; root tip metaphase, with chromosomes distinctly larger than those in preceding genera (fig. 48); fourteen chromosomes with median spindle fiber attachments, two with terminal attachments, four subterminal, and two submedian; three pairs of chromosomes were pointed at the tips, suggesting the presence of satellites.

31. *Escobaria runyonii* Br. & R. (15303): Diploid, twenty-two; eleven pairs of chromosomes clear at diakinesis (fig. 49).

32. *Dolicothele longimamma* (DeCandolle) Br. & R. (1540): Diploid, twenty-two; the usual eleven split chromosomes at metaphase II (fig. 50); chromosomes show wide range in size, one pair large, four medium, six small.

33. *Neomammillaria heyderi* (Muhlenpfordt) Br. & R. (15286): Diploid, twenty-two; diakinesis shows eleven pairs of chromosomes (fig. 51).

34. *Neomammillaria hemisphaerica* (Engelmann) Br. & R. (15287): Diploid, twenty-two; diakinesis, eleven pairs of chromosomes (fig. 52); this species is closely related to *N. heyderi*.

35. *Neomammillaria magnimamma* (Haworth) Br. & R. (16164): Diploid, twenty-two; root tip metaphase (fig. 53); two chromosomes have attachments submedian and appear to carry a satellite.

36. *Neomammillaria compressa* (DeCandolle) Br. & R. (1520): Diploid, forty-four; pollen mother cells at interkinesis show twenty-two pairs of chromosomes; chromosomes give evidence of lagging and non-disjunction; material in greenhouse failed to set fruit.

37. *Neomammillaria chinocephala* (Purpus) Br. & R. (16172): Diploid, twenty-two; eleven pairs of chromosomes at diakinesis (fig. 54); a fragment appeared at diakinesis in addition to the normal set; material scant, complete history not available.

38. *Neomammillaria aureiceps* (Lemaire) Br. & R. (1541): Diploid, twenty-two; prophase II shows eleven split chromosomes in characteristic H shapes (fig. 55).

39. *Neomammillaria multiceps* (Salm-Dyck) Br. & R. (1538 and 15201): Diploid, forty-four; diakinesis shows twenty-two pairs of chromosomes (fig. 56). Two distinct forms of this species are under cultivation at the University of Michigan Botanical Garden. The larger of them, which is fully three times the size of the other and which has certain definite peculiarities of spine color and arrangement, was collected in the lower Rio Grande Valley and was also present in the collection from the Missouri Botanical Garden. The smaller form was also collected in the lower Rio Grande Valley. Both of them are tetraploid with identical behavior in meiosis. Diakinesis (fig. 56) shows a close association of the chromosomes in pairs with no evidence of groupings in fours. In several hundred cells examined, only one showed four homologues together. Separation after metaphase I is prompt and complete (fig. 57). The split preparatory to second division is not so well defined in early anaphase I as it is in normal diploids. The prophase of the second division is entirely normal. The chromosomes are so small that no distinguishing characteristics can be noted in either set.

40. *Neomammillaria decipiens* (Scheidw.) Br. & R. (7546): Diploid, twenty-two; metaphase II shows eleven split chromosomes (fig. 59).

41. *Neomammillaria longicoma* Br. & R. (16161): Diploid, twenty-two; eleven pairs of chromosomes at diakinesis (fig. 60).

42. *Neomammillaria tenampensis* Br. & R. (16151): Diploid, twenty-two; prophase II, eleven split chromosomes (fig. 61); interesting because of similarity to *N. aureiceps*.

43. *Neomammillaria minuta* Bartlett unpub. man. (13756): Diploid, twenty-two; prophase II shows eleven split chromosomes (fig. 62); pollen mother cells smaller than average.

44. *Epiphyllum strictum* (Lemaire) Br. & R. (13901): Diploid,

twenty-two; metaphase II (fig. 63); chromosomes much smaller than average.

45. *Epiphyllum ackermanni* Haworth (12737): Diploid, twenty-two; diakinesis, eleven pairs of chromosomes (fig. 64); the form is interesting because it is generally supposed to be a hybrid.

46. *Rhipsalis mesembrianthemoides* Haworth (7539): Diploid, twenty-two; root tip metaphase (fig. 65); chromosome complement very simple; short chromosomes with either terminal or median attachments.

### Discussion

In the past decade much has been written on the relation of chromosome complements to taxonomy. TISCHLER (27) discusses the conditions which have been reported and classifies them as follows:

1. The *Pinus* type, in which all species studied in a given family have a uniform chromosome count.

2. The *Chrysanthemum* type, in which species within a genus present a straightforward polyploid series.

3. The *Carex* type, genera with species presenting chromosome numbers or series of numbers apparently unrelated.

4. The *Antirrhinum* type, in which each genus has a basic number which is strictly followed but in which each genus is separated from those closely related to it by the difference of one chromosome.

JÖRGENSEN (18) arranges types to include (a) genera in which all species studied have the same number; (b) genera with aneuploid numbers; (c) genera with numbers in multiple relations. It has become increasingly evident as more material is handled that many exceptions will be found to such classifications.

The cacti seem likely to fall chiefly into the first group of both JÖRGENSEN and TISCHLER, in that all of the twenty-one genera here considered contain the basic number of eleven, as do those reported elsewhere. One genus, *Mediocactus*, is represented so far only by a tetraploid, but the evidence indicates that in this case eleven is also the basic number. At the same time there is evidence (24) of a well developed polyploid line in *Opuntia*. SUGIURA (25) reports a haploid count of twelve for two species in two genera. STOCKWELL records a haploid number of nine for *Neomammillaria applanata* (Engelmann) Br. & R. The writer finds one aneuploid (*Echinocereus*

*blanckii*) and three tetraploids (*Neomammillaria multiceps*, *N. compressa*, *Mediocactus coccineus*). Briefly, these constitute the exceptions to the general classification.

As has been suggested, the tribe Opuntieae may be considered more primitive than the Cereeae. It is interesting to note in this connection that so far no basic number other than eleven has been found in the former tribe, and that the only aneuploids reported fall in the Cereeae.

The Cereeae, with which this paper is primarily concerned, contains several scattered tetraploids as well as certain aneuploids. The information available does not permit more than conjecture as to their origin, but certain observations can be made concerning their behavior and position.

*Mediocactus coccineus*, which is the only tetraploid reported from the subtribe Hylocereaneae, is a relatively primitive type. It is a climbing form found in Brazil and Argentina. There are four chromosomes which are alike in shape and size and which appear to bear satellites. This suggests that the plant is a possible autotetraploid (fig. 34).

*Neomammillaria multiceps* is a tetraploid (fig. 56) in which the chromosomes at meiosis are so nearly alike that it has not been possible to differentiate them. As has been stated, the chromosomes are present in pairs at diakinesis. Only rarely do they appear in fours. The view has been generally held that the chromosomes of autotetraploids associate in fours with a fair degree of regularity, and such behavior has been reported in *Aucuba* (21), *Datura* (2), *Hycinthus* (8), *Solanum* (19, 20), *Primula* (9), and *Prunus* (7). On the other hand, DAVIS (10), in describing an *Oenothera* known to be autotetraploid, reports that the chromosomes do not form groups of four but are in pairs at diakinesis. It has been suggested that autotetraploid plants may in time undergo sufficient stabilization through differentiation of homologous chromosomes so that they become digenomic. The behavior suggests that this may have occurred here, since there is only one species to which *Neomammillaria multiceps* is obviously closely allied (*N. prolifera*), and there is no evident way by which it could have arisen through hybridization. Dr. ELZADA CLOVER of the University of Michigan Botanical Garden has secured

germination of 90 per cent or more from seeds of different plants of this species. The range of *N. multiceps* is not great. It is found from the coast to approximately 100 miles up the Rio Grande River valley and a short distance south into Mexico.

The third tetraploid, *N. compressa*, shows pairing to a large extent at diakinesis, but metaphase I is often disturbed and a few univalents occur. It flowers freely but has not set fruit in the greenhouses of the University. This species is common in Mexico, and has a number of well recognized varieties which suggest a series of forms similar to that reported for *Opuntia polyacantha* (24).

The only aneuploid discovered in the present material is *Echinocereus blanckii*, with a haploid number of twelve. As was pointed out, this species is very similar to *E. pentalophus*, except that (a) the flower is a uniform mallow purple, lacking the white center of *E. pentalophus*; (b) the plant is characteristically 5-6 ribbed rather than 4-5; (c) the ribs have more prominent papillae; (d) the plants have heavy tuberous roots which are not found in the other species. Both species are found in the region of the lower Rio Grande River valley, although *E. blanckii* is distinctly more limited in range. It is possible that this species arose from *E. pentalophus* through the addition of one chromosome by non-disjunction. There are many cases of aneuploidy on record, among them members of *Primula* (4), *Viola* (6), *Crepis* (22), *Antirrhinum* (13), *Lactuca* (15), and *Carex* (11, 12). Aneuploidy may be expected in a group as large as the Cactaceae.

Satellites have been reported in the Cactaceae by JOHANSEN (17) and STOCKWELL (24). The writer found satellites in *Selenicereus hondurensis*, *Mediocactus coccineus*, *Echinocereus blanckii*, and *Neomammillaria magnimamma*.

Primary constrictions appear with varying degrees of clarity in somatic chromosomes. Usually they are clearly indicated by a bend in the chromosome. An attempt has been made to group the chromosomes on the basis of primary constrictions. The random sampling here reported is not sufficient to present an accurate picture of chromosome morphology in the Cereae, but it does suggest some possibilities of relationships in chromosome configuration.

The facts that the basic number eleven is so consistently present



in all of the genera studied, and that it is the haploid number for the majority, suggest strongly that evolution in this family has been accomplished largely through gene mutations. The two factors which appear to rank next in importance in the development of the family are polyploidy and hybridization.

From the available information, the Cereaceae do not seem to show as strong an inclination toward polyploidy as do the Opuntieae. Such polyploids as do occur in the Cereaceae may arise through somatic doubling. There appears to be a stronger tendency in this tribe to develop aneuploids, possibly owing to the fact that the genera are more divergent than in the Opuntieae.

The evidence seems to show that the family is still relatively young, in that it is known to be giving rise to numerous new types.

### Summary

1. The cytological work on record is briefly surveyed. There are reports on only nineteen species. Work has been confined to members of the tribe Cereaceae, which is the most highly evolved tribe in the family and contains the greatest number of genera.

2. The chromosome numbers are given for forty-six species, including two forms and one hybrid. The basic number, eleven, is present in every genus here reported, and is the haploid number in the majority of species.

3. Three tetraploids are recorded and discussed as to origin. Of these, *Mediocactus coccineus* is one of the simpler Cereaceae and the others, *Neomammillaria compressa* and *N. multiceps*, are both members of the largest genus in the tribe.

4. One aneuploid species, *Echinocereus blanckii*, is described. It is suggested that it arose out of *E. pentalophus* by reduplication of one chromosome through non-disjunction.

5. Meiosis of *Echinocereus papillosus* was studied. The behavior is reported as entirely normal. The prophase threads associate by parasynapsis.

6. Certain bodies in the cytoplasm are described as they occur in *E. papillosus* and a majority of the other species studied. They can commonly be seen in early pachynema as two oval structures lying at either end of the cell. Their staining reactions become more in-

tense as prophase advances and is greatest just before telophase I. At this time or shortly after, two other such bodies appear which come to lie between the two nuclei. At the end of second division there is one body beside each nucleus. Often eight bodies are found at the end of telophase II.

7. The relation of the chromosome numbers to taxonomy is discussed, and it is pointed out that the rather simple chromosome situation is in keeping with the morphological evidence that the tribe Cereeae is young.

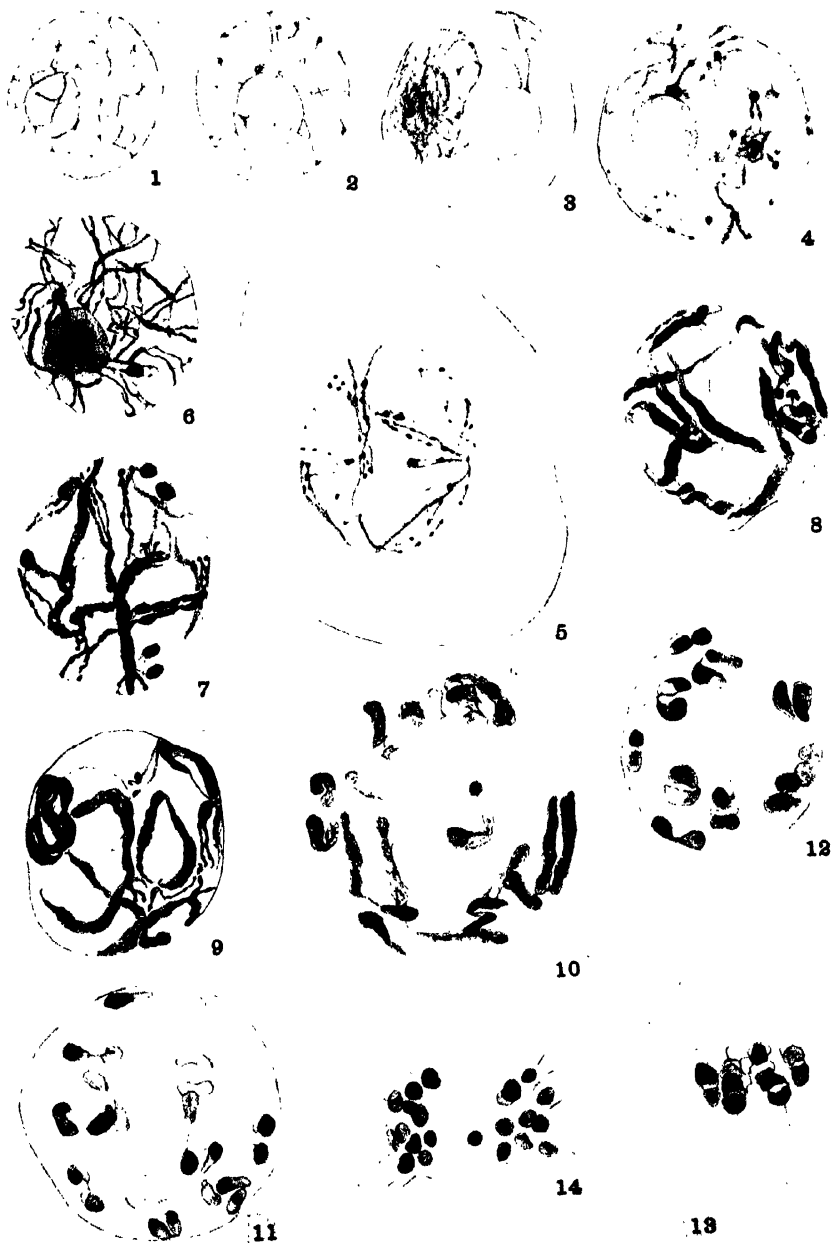
The writer expresses to the Botanical Garden of the University of Michigan her gratitude for assistance which made this study possible. It is with pleasure that I acknowledge indebtedness to Professor BRADLEY M. DAVIS for his interest in the progress of the work and his helpfulness in the preparation of this paper. Thanks are also due Dr. ELZADA CLOVER for the determination of material and the Shiner Cactus Nursery, Laredo, Texas and the Rio Grande Valley Cactus Garden, Edinburg, Texas for their cooperation in making available certain rare material.

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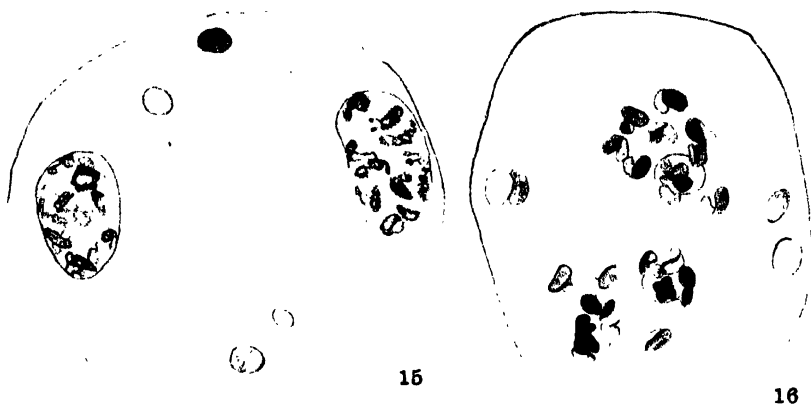
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BEARD on CACTACEAE







## EXPLANATION OF PLATES I, II

All figures were drawn with the aid of a camera lucida under Zeiss apochromatic objective 1.5 (120 $\times$ ) in combination with the ocular K 20 $\times$ . Measurements were made with an eyepiece micrometer in the Zeiss K 10 $\times$  ocular.

*Echinocereus papillosus* Linke

## PLATE I

FIG. 1.—Resting nucleus from archesporium.

FIG. 2.—Late leptonema.

FIG. 3.—Synzesis.

FIG. 4.—Early pachynema, parallel threads evident.

FIG. 5.—Middle pachynema, appearance of cytoplasmic bodies.

FIG. 6.—Zygonema conspicuous.

FIG. 7.—Late pachynema, appearance of four closely associated threads.

FIG. 8.—Chromosome segments defined.

FIG. 9.—Diakinesis, two chromonemata visible.

FIG. 10.—Diakinesis.

FIG. 11.—Late diakinesis.

FIG. 12.—Diakinesis, condensation practically complete.

FIG. 13.—Metaphase I.

FIG. 14.—Anaphase I, chromosomes beginning to show split preparatory to second division.

## PLATE II

FIG. 15.—Interkinesis, four bodies present in cytoplasm.

FIG. 16.—Prophase II, four bodies in cytoplasm, nucleolus also present.

FIG. 17.—Metaphase II.

FIG. 18.—Anaphase II.

FIG. 19.—Telophase II, eight bodies in cytoplasm.

FIG. 20.—Tetrad showing cleavage by furrowing.



# MECHANISM AND QUANTITATIVE APPLICATION OF THE PEA TEST

J. VAN OVERBEEK AND F. W. WENT

(WITH EIGHT FIGURES)

## Introduction

Some years ago WENT (9) published a new method for determining the growth hormone (auxin) concentration of solutions, the so-called pea test. If the rapidly elongating region of an etiolated pea stem is split lengthwise and placed in water, the two halves will bend outward owing to tissue tension. If the split stems are placed in solutions containing auxin, however, the growing zones will bend inward, assuming an S or crescent outline. The inward curvature is proportional to the log of the auxin concentration. The advantages of the pea test over the standard *Avena* test can be summarized as follows:

1. Large range of concentrations in which the effect is a function of the concentration.
2. Humidity need not be constant.
3. Curvature reaches a maximum in the course of 4 to 12 hours and does not change thereafter, so that the test can be measured at any time between 12 and 60 hours after the beginning of the experiment.
4. No lack of sensitivity, if occasionally exposed to small amounts of white light.

The pea test, however, has certain disadvantages as compared with the *Avena* test, since (a) it requires about 20 cc. of the active solution whereas the *Avena* test can be carried out with less than 1 per cent of this amount; (b) it is less specific in its reaction, so that a number of substances which in the *Avena* test give only a very slight reaction, and then only in a very high concentration, are active in the pea test; (c) it is not so quantitative as the *Avena* test.

Point (b) has been turned into a distinct advantage. THIMANN (8) and HAAGEN-SMIT and WENT (4) have shown that substances,

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hardly active in the *Avena* test but active in the pea test, would induce growth in length in short sections of *Avena* coleoptiles if suspended in a solution of those substances. They showed therefore that those particular substances were merely lacking the ability of being transported in the plant. Based on the more general response of the pea test, a completely new attack on the problem of the relation between chemical structure and physiological activity was made possible (6). Point (c) will be considered in the last part of this paper.

The only recent paper dealing with the mechanism of the pea test is one by JOST and REISS (5). In it they bring out the following points: 1. The pea test does not work so consistently as it should, so that often no curvatures at all are obtained. This must be attributed to unsuitable pea varieties and/or culture conditions. With Alaska peas there is some variation in sensitivity from day to day, but the test is always positive. 2. The tentative explanation given by WENT (9) is erroneous. It will be seen that we have come to the same conclusion. 3. *Taraxacum* flower stalks, like the pea stem, give auxin curvatures ("Wentreaktion") after being split lengthwise. Only when the stems are deficient in auxin they react and the reaction is caused by the increased growth of the outside.

### Mechanism of pea test

Alaska peas were soaked in water for a few hours and then planted in moist sand. From then on the plants were grown in a physiological darkroom with controlled temperature and humidity (24° C. and 90 per cent respectively). All the experiments were performed in the darkroom under the same conditions. About one week after the seeds had been planted the pea seedlings were ready for use. From a number of uniform plants the terminal buds (about 5 mm. in length) were cut off. The upper 5 cm. of each remaining stump was used for the experiments. Such a piece consisted of an internode without the nodes. The stems were then marked into segments 1 mm. in length. This was done by placing them on the inked wires of a marking device<sup>1</sup> until at least half of the circumference showed thin parallel ink lines 1 mm. apart.

<sup>1</sup> The marking device consisted of a number of thin copper wires (0.005 of an inch in diameter) which were stretched parallel and 1 mm. apart on a brass frame. India

After the stems were marked, the apical 3 cm. was split lengthwise into two equal halves in such a way that both halves were plainly marked. The markings permitted an accurate measurement of the growth of the 1 mm. long segments at the wounded side (inside) and at the epidermis side (outside), even if the halved stems were strongly curved. Next the marked and split stems were put into petri dishes. Each dish contained 25 cc. solution and received three split stems (six halves). The stems were left in the solutions for about 18 hours, after which time they were removed from the darkroom and the length of the segments measured with an eye piece micrometer.

#### PEA TEST CURVATURE CAUSED BY DIFFERENTIAL GROWTH

The results of the experiments described in the previous section are summarized in table 1 and figure 1. The stems were put in tap water and in five different solutions of indole-3-acetic acid (hetero-auxin). The lowest concentration used contained 6.57 mg. of the hormone per 100,000 cc. of water. Such a concentration will give a curvature of about  $6^\circ$  in the standard *Avena* test. Table 1 gives the increase in length of the upper 25 mm. of the stems. In the table this 25 mm. has been subdivided into five zones, each having an initial length of 5 mm. The increase in length of these zones represents the sum of the increases of five segments of an original length of 1 mm. each. It may be seen that the upper 15 mm. of the stems represents the main growing region. Figure 1 shows that both the outside and the inside of the halved stems grow. In water (O on the abscissa) the inside grows somewhat more than the outside. This results in a negative traumatotropic curvature. In a concentration of 0.0657 mg. auxin per liter, the growth of both the inside and the outside is increased as compared with water. The inside grows slightly less than the outside. This difference in growth (curve 0-1) causes the stems to curve positively. In still higher concentrations the growth of the outside increases with the concentration, whereas the growth of the inside decreases. This is probably not caused by a direct action of

ink was sparsely applied to the wires with a soft brush. An even marking over the entire stem was obtained by gently pressing it upon the wires by means of a flexible piece of rubber tubing. When the tubing was rotated the pea stem rolled over the wires and the ink marks showed clearly over at least half the surface of the stem.

the auxin on the growth of the inside, but is a mechanical effect due to the bending of the stem. For comparison, the growth of non-split stems is also given. Their growth increases with increasing concentration, and has in every instance a higher value than the outside of the split stems.

TABLE 1

GROWTH OF INSIDE AND OUTSIDE OF SPLIT AND OF NON-SPLIT PEA STEMS IN AUXIN SOLUTIONS. STEMS DIVIDED INTO FIVE SEGMENTS, EACH WITH INITIAL LENGTH OF 5 MM. FIGURES GIVE INCREASE IN LENGTH IN 0.1 MM. AND ARE AVERAGES OF 24 SPLIT STEMS AND 12 NON-SPLIT ONES. MEASURED 18 HOURS AFTER PLACING IN SOLUTIONS. EXPERIMENT NOS. 60714, 60715, 60718, AND 60721

SOLUTION	STEM	DISTANCE OF ZONE BELOW TOP				
		0-5 MM.	5-10 MM.	10-15 MM.	15-20 MM.	20-25 MM.
Water	Non-split	4.7±0.8	6.2±0.7	4.5±0.5	4.3±0.4	2.3±0.4
	Inside	2.9±0.4	3.5±0.3	2.6±0.2	2.9±0.2	2.0±0.3
	Outside	1.5±0.3	1.5±0.3	0.6±0.2	0.2±0.3	0.1±0.3
6 57 mg. hereto- auxin per 100,000 cc.	Non-split	14.3±1.1	13.0±1.2	9.2±1.1	6.0±0.9	4.3±0.6
	Inside	9.7±0.7	7.7±0.7	5.6±0.5	4.3±0.4	2.6±0.3
	Outside	9.7±0.7	8.9±0.7	6.7±0.6	4.2±0.7	1.9±0.6
6 57 mg. hetero- auxin per 40,000 cc.	Non-split	23.3±2.4	18.1±1.5	10.8±1.3	7.6±0.8	3.9±0.7
	Inside	9.5±0.9	6.0±0.6	5.4±0.5	4.7±0.4	3.3±0.4
	Outside	12.6±0.8	11.9±0.8	8.1±0.8	5.8±0.8	2.3±0.5
6 57 mg. hetero- auxin per 20,000 cc.	Non-split	21.5±2.2	17.3±1.2	10.6±0.6	7.2±0.7	3.9±0.7
	Inside	8.5±1.1	6.5±0.8	5.9±0.4	4.1±0.3	3.2±0.4
	Outside	15.3±0.7	14.8±0.6	9.7±0.8	6.8±0.8	3.2±0.6
6 57 mg. hetero- auxin per 10,000 cc.	Non-split	26.4±2.0	23.2±1.6	14.1±1.1	7.3±0.8	3.8±0.8
	Inside	5.9±0.8	4.0±0.5	5.1±0.4	4.8±0.3	3.4±0.5
	Outside	20.8±1.2	16.7±1.1	11.4±1.1	7.2±1.2	3.0±0.5
6 57 mg. hetero- auxin per 4000 cc.	Non-split	26.9±2.0	22.9±1.9	16.4±1.6	8.5±1.3	5.0±1.0
	Inside	5.9±0.8	5.2±0.7	5.8±0.5	4.4±0.4	3.8±0.4
	Outside	24.7±1.5	23.0±1.1	16.4±0.8	7.0±0.8	3.0±0.6

After the completion of the experiments just described the article by JOST and REISS (5) appeared, in which these workers showed that upon immersion in an auxin solution the inside of a split *Taraxacum* stem grows less than its outside. Their results are in agreement with the facts presented here.

## PREVENTION OF AUXIN UPTAKE THROUGH WOUNDED SURFACE

After it had been established that the inside grows less than the outside, the question rose as to the reason for this. The first experiment was as follows. Pea stems were split in the usual way and their bases inserted in vials of water. The apical split ends were in humid air under a bell jar in the darkroom. On the split end a lanolin-auxin paste was smeared in such a way that the paste covered either the

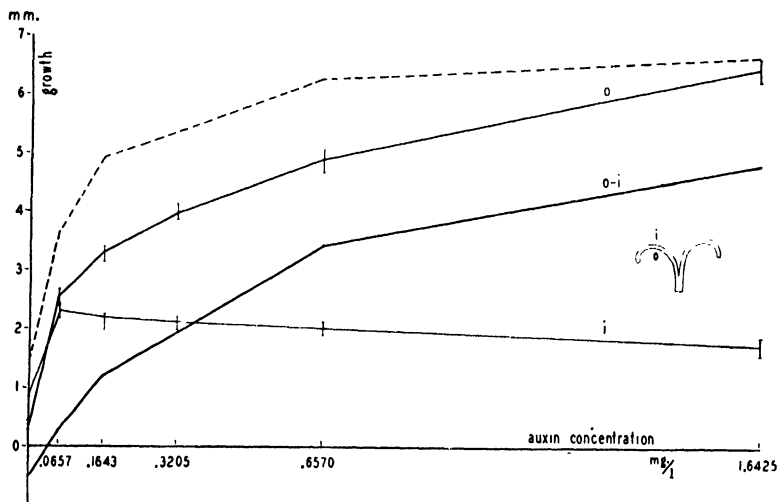


FIG. 1.—Increase in length of upper 15 mm. of inside (i) and outside (o) of halved pea stems and of non-split (broken line) pea stems in auxin solutions. Abscissa: concentration of indole-3-acetic acid in mg. per liter. Ordinate: increase in length in mm. during 18 hours. Small vertical lines indicate range of probable errors. Difference between growth of outside and inside indicated by o-i, which curve corresponds to actual "pea test curvature."

inside, the outside, or the apical ends of the halved stems. About 18 hours later the curvatures were measured. The results of this experiment are given in table 2. If no paste was applied the halves curved slightly negatively. In all cases application of the paste containing auxin resulted in a positive curvature. The largest curvature was obtained when the paste was applied to the outside; the smallest when the auxin was applied to the inside. A distinctly positive curvature was also obtained if the paste was applied to the apical ends of the halves. This would indicate that the response of the outside is greater than that of the inside.

In order to check this possibility, the following experiment was conducted. The epidermis of pea stems was removed and sections of stem 6.4 mm. long were cut. These were put in an auxin solution.

TABLE 2

CURVATURES OF SPLIT PEA STEMS WITH BASES INSERTED IN VIALS  
CONTAINING WATER AND SPLIT ENDS IN VERY HUMID AIR

EXPERI- MENT NO.	PERCENT- AGE CON- CENTRA- TION OF AUXIN PASTE	PASTE ON OUTSIDE	NO. OF HALVES	PASTE ON INSIDE	NO. OF HALVES	PASTE ON TOP	NO. OF HALVES	NO PASTE APPLIED	NO. OF HALVES
60725	0.02	$+304 \pm 41$	5	$+86 \pm 14$	6	$+76 \pm 9$	12	.....	
61116.	0.0036	$+185 \pm 19$	20	$+17 \pm 10$	20	$+53 \pm 8$	20	$-19 \pm 4$	20

TABLE 3

GROWTH OF SECTIONS OF PEA STEMS IN AUXIN SOLUTIONS. INITIAL LENGTH  
6.4 MM. EPIDERMIS OF HALF OF SECTIONS REMOVED. AVERAGES  
OF 6 SECTIONS. EXPERIMENT NO. 61119

SOLUTION		INCREASE IN LENGTH IN PERCENT- AGE OF INITIAL LENGTH	
		AFTER 24 HOURS	AFTER 18 HOURS
Distilled water	{ With epidermis	9	17
	{ Without epidermis	15	22
0.03 mg. auxin per liter	{ With epidermis	16	28
	{ Without epidermis	16	22
0.3 mg. auxin per liter	{ With epidermis	22	39
	{ Without epidermis	14	22
3 mg. auxin per liter	{ With epidermis	23	47
	{ Without epidermis	12	25

In order to allow sufficient oxygen uptake, the sections were barely covered with the solution and placed in an electric shaker. The whole experiment took place in the darkroom.

The results of one experiment are given in table 3. In tap water the increase in length is more in the sections without epidermis than

in the ones with epidermis. In the auxin solutions, on the contrary, the sections with the epidermis left intact grow considerably more than the ones with the epidermis removed. The sections in water elongate because they contain a certain amount of auxin which is naturally present in the plant. The increase in length caused by this native auxin is 22 per cent in sections without epidermis, after 18 hours. In cases where the sections without epidermis have been immersed in auxin solutions the increase in length over the same period of time is also 22 per cent, except for the highest concentration where it is 25 per cent. This strongly indicates that the sections from which the epidermis has been removed do not take up hormone from the solution but grow with the hormone they contained at the time the sections were cut. Figure 2 presents the results of a similar but more elaborate experiment. The increase in length of the sections is plotted against time. The curves of the sections without epidermis in water and with epidermis in water run nearly parallel. This means that their growth rate is the same, showing that the response to auxin of the wounded sections (without epidermis) is the same as that of the sections with the epidermis left intact. Furthermore, it is clear from this figure that the growth rate of the sections with epidermis in auxin solution is considerably greater than that of the sections without epidermis in auxin solution. The growth rate of the latter is but slightly more than that of the sections in water. From the growth of the sections in water it was concluded that the response to auxin is the same in sections with and without epidermis. Hence, from the difference in growth rate between the sections with and without epidermis in auxin solutions it may be concluded that removal of the epidermis (wounding) prevents the growth substance from being taken up.

Experiments such as just described for the pea were repeated with coleoptile sections of *Avena*. The primary leaf was not removed, in order to prevent auxin from being taken up through the intact inner epidermis of the coleoptile. The results of one such experiment are presented in figure 3. It leaves no doubt that the growth rate of sections of *Avena* coleoptiles, if treated as described, is considerably less if the epidermis is removed than if it is left intact. Using sections of *Avena* coleoptiles, BONNER (1, 2) found that removal of the outside

epidermis increased the growth of such sections in auxin solutions. Auxin taken up through the inner epidermis (BONNER removed the

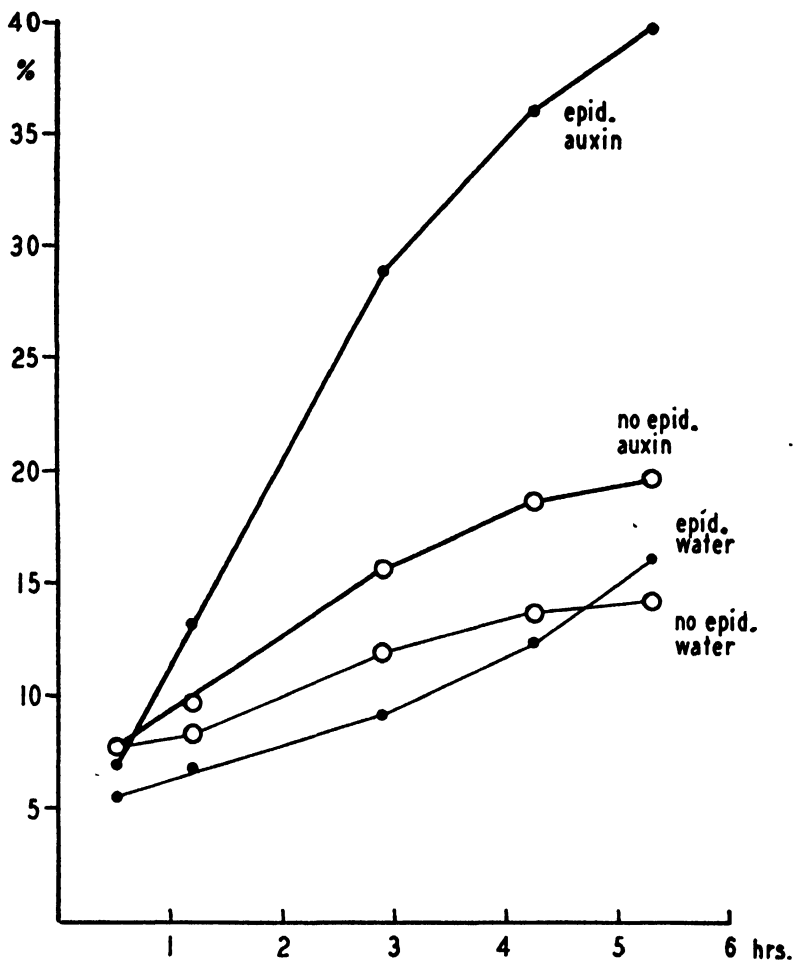


FIG. 2.—Increase in length of sections of pea stems with and without epidermis in water and in a solution containing 2.93 mg. indole-3-acetic acid per liter. Abscissa: time after sections were placed in solution. Ordinate: increase in length in percentage of original length (6.4 mm.). Experiment no. 61208.

primary leaf) must be one of the reasons why his results do not agree with ours.

A direct proof that sections from which the epidermis has been



removed cannot grow because the hormone cannot enter was made by infiltrating these sections with auxin and letting them grow again (table 4). The sections were infiltrated by sucking air out of the intercellular spaces and replacing this by an auxin solution. This was

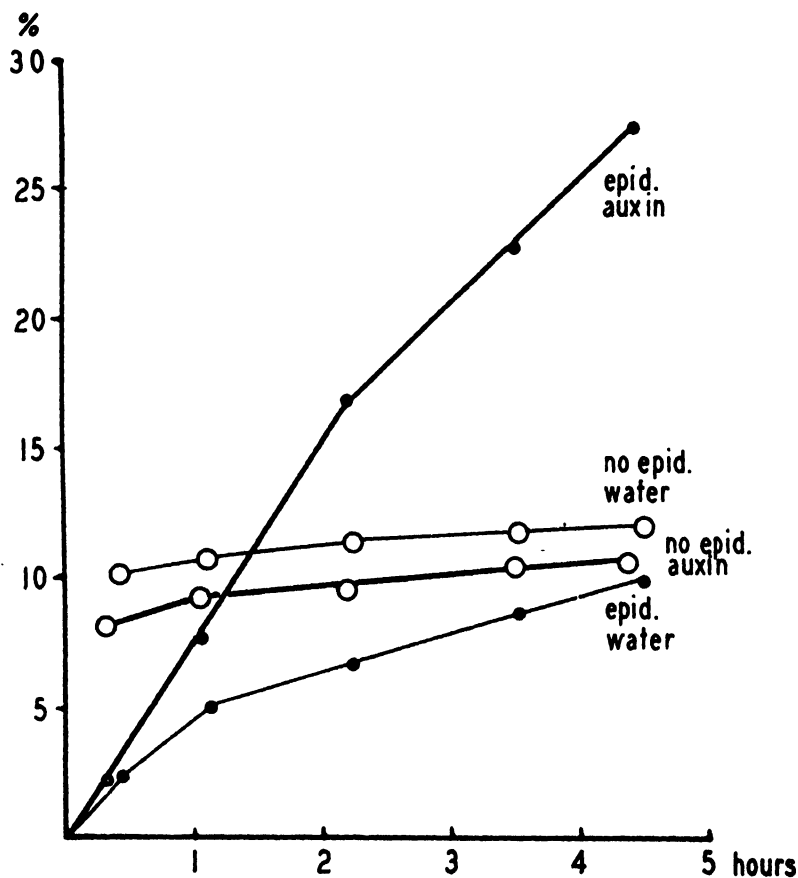


FIG. 3.—Increase in length of sections of *Avena* coleoptiles with and without epidermis in water and in solution containing 2.93 mg. indole-3-acetic acid per liter. Abscissa and ordinate same as in fig. 2. Experiment no. 61207.

done by evacuating the space above the auxin solution containing the sections and then allowing the air to enter again. Removal of the epidermis does not greatly influence growth. It is not even necessary to fill the intercellular spaces with auxin solution. Infiltration with

tap water followed by immersion of the sections in auxin solution has a similar effect. Table 5 shows that the sections of which the epidermis had been removed show the same increase in length when infiltrated with auxin and kept in an auxin solution, as they do when

TABLE 4

GROWTH OF INFILTRATED SECTIONS OF PEA STEMS. INITIAL LENGTH 6.4 MM. AVERAGES OF 12 SECTIONS.  
EXPERIMENT NO. 61126b

		INCREASE IN LENGTH IN PERCENTAGE OF INITIAL LENGTH AFTER				
		50 MIN.	2 HR.	3 HR. 45 MIN.	10 HR.	22 HR.
Infiltrated with water	With epidermis	1.6	3.9	9.5	18.8	21.7
	Without epidermis	3.6	7.4	10.6	17.3	19.5
Infiltrated with 3 mg. auxin per liter	With epidermis	2.5	9.8	18.0	24.8	40.0
	Without epidermis	6.0	10.6	18.5	31.0	37.3

TABLE 5

GROWTH OF INFILTRATED SECTIONS OF PEA STEMS WITH EPIDERMIS REMOVED. INITIAL LENGTH 6.4 MM. AVERAGES OF 12 SECTIONS.  
EXPERIMENT NO. 61202

	INCREASE IN LENGTH IN PERCENTAGE OF INITIAL LENGTH AFTER				
	15 MIN.	2 HR. 15 MIN.	4 HR. 15 MIN.	9 HR. 30 MIN.	20 HR. 30 MIN.
Infiltrated in water and kept in 3 mg. auxin per liter.....	7.0	11.2	16.1	22.8	35.6
Infiltrated with 3 mg. auxin per liter and kept in this solution.....	8.3	14.8	20.0	26.5	33.4

infiltrated with water followed by immersion in an auxin solution.

Next the knowledge obtained from the experiments with and without epidermis was applied to the case of the split pea stems. Here also a wound is made by splitting the stems lengthwise, and

similarly it was to be expected that auxin could not enter through this wound into the stem. Split pea stems were infiltrated with an auxin solution or infiltrated with water and later immersed in an auxin solution. The result was that split stems either remained straight or showed a reversed pea test curvature (negatively traumatotropic). In order to prove definitely that the inside resumes growth upon infiltration, the stems were marked into segments 1 mm. long and the increase in length measured in a way similar to

TABLE 6

INCREASE IN LENGTH OF INSIDE AND OUTSIDE OF INFILTRATED SPLIT PEA STEMS DIVIDED INTO FIVE SEGMENTS, EACH HAVING INITIAL LENGTH OF 5 MM. FIGURES GIVE INCREASE IN LENGTH IN 0.1 MM. AND ARE AVERAGES OF 16 HALVES. MEASURED 18 HOURS AFTER INFILTRATION. EXPERIMENT NO. 61204

		DISTANCE OF ZONE BELOW TOP				
		0-5 MM.	5-10 MM.	10-15 MM.	15-20 MM.	20-25 MM.
In 0.3 mg. auxin per liter	Inside	19.6 ± 0.8 (8.7)*	18.6 ± 0.8 (6.4)	16.3 ± 1.0 (5.8)	11.5 ± 1.0 (4.2)	8.4 ± 1.1 (3.2)
	Outside	17.5 ± 0.8 (14.8)	14.5 ± 1.0 (14.2)	12.0 ± 0.9 (9.4)	8.8 ± 0.9 (6.6)	4.3 ± 0.9 (3.0)
Infiltrated with distilled water	Inside	7.4 ± 0.6 (2.9)	7.0 ± 0.5 (3.5)	6.6 ± 0.5 (2.6)	5.2 ± 0.5 (2.9)	4.6 ± 0.4 (2.0)
	Outside	3.4 ± 0.5 (1.5)	3.2 ± 0.4 (1.5)	2.4 ± 0.4 (0.6)	1.5 ± 0.4 (0.2)	1.1 ± 0.3 (0.1)

\* Figures in parentheses are values for increase in length of non-infiltrated split pea stems, obtained by interpolation from table 1.

that described earlier in this paper. Table 6 shows the results of this experiment. In every instance the growth of the inside was greater than that of the outside. The growth of the outside was about the same whether the split stems were infiltrated with auxin or not.

One would expect that in high auxin concentrations the auxin entering from the epidermis side would reach the wounded side and thus increase the growth on this side. This is probably the case in very high concentrations, as may be deduced from figure 6. There was also the possibility that the same agent which prevented the hormone from entering the plant through the wound also would reject the auxin from the wound inside the plant and thus cause an

unequal distribution of the hormone. In sections which were wounded on one side, however, such an unequal distribution could not be found.

### Quantitative application of pea test

#### TECHNIQUE

As suggested in a previous paper (9), the pea test can easily be adapted to quantitative auxin determinations. When Alaska peas are soaked in tap water for 6-8 hours, planted in wet sand and placed in a darkroom at 24°C. to germinate, the seedlings reach a length of about 12 to 15 cm. in the course of seven days. A rigid selection is then carried out; plants too short and too long are eliminated, and

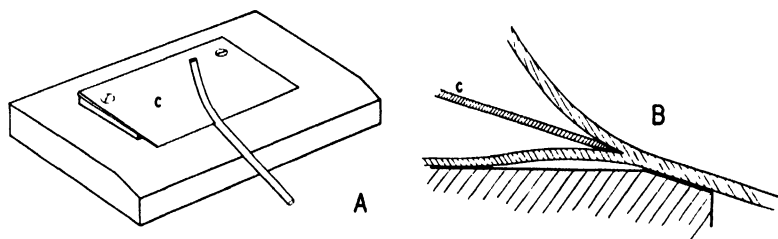


FIG. 4.—Cutter (A) and section through the instrument (B) showing position of razor blade (c).

only those shoots are used in which the third internode (between the highest scale or bract and the lowest leaf) is at least 40 mm. long, and the fourth internode not more than 5 mm. long. The apical 5 mm. of the stem is cut off and the stem split downward from the apical cut into two equal halves. For a uniform response it is essential to have the cut exactly median. This can be done either by hand or by means of a simple cutter (fig. 4). The split should be about 1-2 cm. longer than the growing region. Just below the end of the split the stem is cut and the split halves, which are still attached to each other at their base, are dropped in a large container filled with water to wash off the contents of the cut cells.

The solutions to be tested are made in a series of dilutions (successive concentrations differing from two to ten times), 20 cc. of which is poured in petri dishes 12 cm. in diameter. After rinsing the cut sections for about two hours, they are transferred to these dishes;

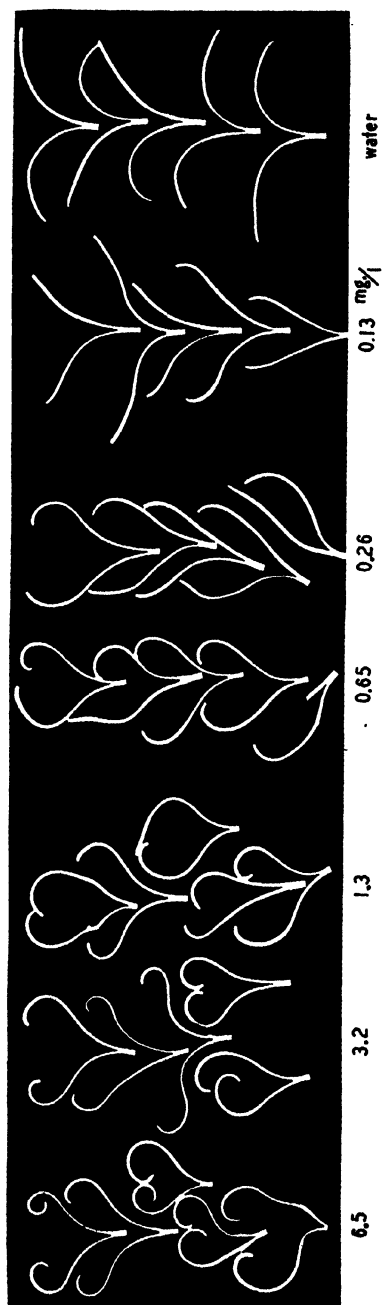


FIG. 5.—Shadow picture of slit pea stems which have been for 18 hours in different concentrations of indole-3-acetic acid, at pH 7.

five to eight, that is ten to sixteen halves, per dish. The halves are arranged so that all parts of each stem touch the solution. Under certain conditions the ends will move out of the solution, in which case it is advisable to invert them while this is happening since otherwise the parts out of the solution do not continue to curve. Within wide limits (20 minutes–6 hours) there is hardly any effect of time of washing upon the subsequent pea curvatures. With short washing periods, however, the peas do not react as uniformly as they should.

After 12 to 60 hours the curvatures are measured directly, or shadow pictures are made on bromide paper (fig. 5) and the curvatures measured later. Measurement is made with a celluloid protractor to which is attached a rotating celluloid disk with parallel lines and pointer. The protractor is first lined up with the straight region of each half (the region where the negative tissue tension curvature of the basal zones changes into the positive auxin curvature of the apical zones). The lines of the upper disk are then made parallel with the tangent of the extreme tip and the angle is read (either directly or after addition of  $n \times 360$ , depending on the number of loops). The curvature plotted against the log of the concentration gives in most cases a straight line. Above a certain concentration the curvatures do not increase further; at very high concentrations they decrease again (fig. 6). The relation between the concentration of a given substance and the curvature it produces can thus be expressed:

$$\alpha = K \log \frac{c}{c_0}$$

in which alpha is the observed curvature,  $c$  the concentration in moles per liter,  $c_0$  the concentration at which the curvature is zero, and  $K$  a constant characteristic for the slope of each line. The value  $c_0$  may be called the minimum active concentration, and denotes the point where the activity curve crosses the O line. This point is generally determined by extrapolation but may also be determined by direct measurement. The value of  $K$  denotes the rate of increase of curvature with increasing concentration. For each substance causing growth these constants are different, and thus a substance can be characterized by them.

## EFFECT OF pH ON PEA TEST CURVATURES

Great care should be taken to adjust all solutions to the same pH, since the auxin curvatures are very different at different pH. Only phosphate and biphthalate buffers were satisfactory; buffers requiring citric acid were unsuitable since the citric acid contained traces of active substances which caused slight bending of the split stems.

This pH effect is obvious even in the control plants in buffer solutions, which show marked differences in their behavior at different

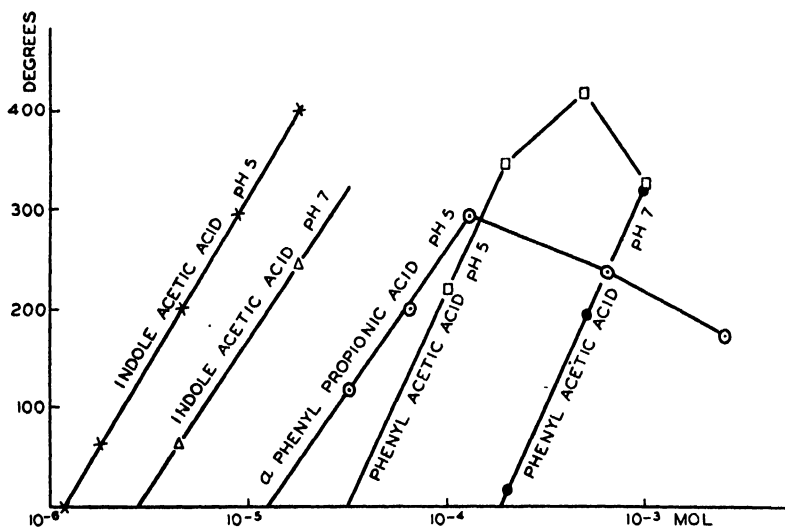


FIG. 6.—Relation between curvature (ordinate) and molar concentration (abscissa) in pea test, for various compounds at different pH.

pH, as shown by the following figures. The angle over which the split ends were bent away from their original position before cutting were  $75^\circ$  at pH 8;  $85^\circ$  at pH 7;  $102^\circ$  at pH 6;  $125^\circ$  at pH 5; and  $255^\circ$  at pH 4. At pH 3 the buffer is toxic, so that although the ends first rapidly curve outward they nevertheless soon lose their turgor. These are the so-called acid curvatures (7, 1). They are in opposite direction from the auxin curvatures, and thus the acid and auxin effects are easily distinguishable. It is the same effect as described by BOUILLENNE (3) in epidermis strips of onion leaves. Another effect of the pH on the pea test is noticed when peas are placed in the

same auxin concentration but at different pH. In concentrations giving a maximal response the effect is hardly perceptible, but in lower concentrations the curvature at a low pH is always greater than that at a higher pH. Figure 6 illustrates this for a number of compounds. The activity of each substance has been determined at pH 5 and 7. Depending upon the specific properties of each substance, the activity is decreased twofold to tenfold by decreasing the acidity from pH 5 to 7. Over the whole range of concentrations the

TABLE 7  
VALUES OF  $C_0$  AND K FOR INDOLE-3-ACETIC ACID  
ON DIFFERENT DAYS

	pH 5		pH 7	
	$C_0$	K	$C_0$	K
1936				
November 12 . . . . .	.....	.....	14.5	130
November 19. . . . .	10	275	15	245
December 1. . . . .	8.3	320	25	295
December 16 . . . . .	10	235	17	190
1937				
January 7.. . . .	12	310	.....	.....
January 14. . . . .	.....	.....	21	255
February 2.. . . .	10	230	.....	.....
February 23.. . . .	5.6	175	14	175
March 5.. . . .	11	345	.....	.....
Mean . . . . .	$9.6 \pm 0.8$	$256 \pm 24$	$17.7 \pm 1.8$	$215 \pm 25$

activity is generally decreased to the same extent for a particular substance. The actual relation between the physical and chemical properties of a given substance and the effect of the pH on its activity in the pea test are under further investigation.

#### DIURNAL AND SEASONAL CHANGES IN SENSITIVITY

There is considerable variation in the sensitivity of peas on different days. The value of K in particular changes. This is the same as in the *Avena* test, in which also diurnal and seasonal variations occur. To give an idea of the variations, table 7 lists the  $C_0$  and K values for indole-3-acetic acid on different days. These variations are not due to a lack of uniformity of the material nor to changes of



temperature, humidity, or darkroom illumination; they depend on as yet uncontrollable external factors. These factors may influence the auxin content of the peas at the moment of splitting, the uptake of the active substance, or the sensitivity of the growing cells.

To illustrate the effect of age of the material upon the reaction, an experiment was undertaken in which each day a number of stems

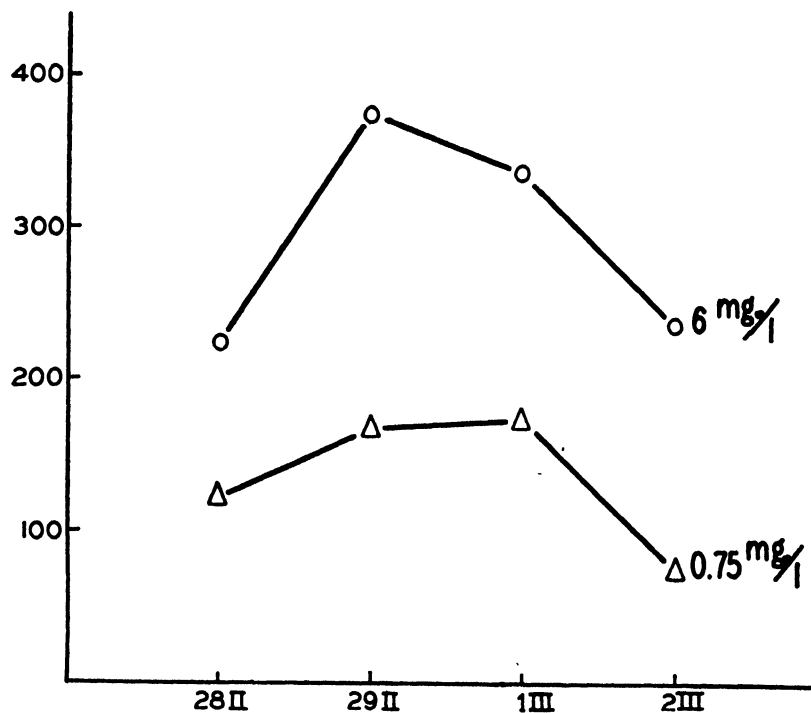


FIG. 7.—Varying sensitivity of peas from same lot on successive days. Ordinate: curvature of pea test, when split stems are treated with 6 or 0.76 mg./l. of indole-3-butyric acid.

were cut from the same set of peas. Figure 7 shows the results. The same peas, or the same internodes even, may give different reactions on successive days. In quantitative tests, therefore, a series of two or three control solutions (preferably of known concentrations of indole-3-acetic acid between 0.5 and 2 mg. per liter) should be included in order to determine the sensitivity of the plants.

## SUITABILITY OF VARIOUS PEA RACES FOR THE TEST

Different races of peas have been tested. Most of these were supplied by Dr. W. BROTHERTON, to whom our thanks are due. Of the fifty-five varieties tested, only two (Extra Early Market and Horal) were completely unsuitable, giving hardly any auxin curvatures. However, this may have been caused by the very poor growth due to infection. Eleven other varieties were not very suitable and gave moderate reactions only (some of them were dwarf varieties). The other forty-two were excellent. It does not seem necessary to list

TABLE 8

PEA TEST CURVATURES OF DIFFERENT VARIETIES IN THREE CONCENTRATIONS OF INDOLE-3-ACETIC ACID; ALL PLANTS 8 DAYS OLD WHEN TESTED

VARIETY	TYPE OF STEM	CURVATURE IN INDOLE-3-ACETIC ACID		
		3 MG./l	1 MG./l.	0.3 MG./l
Alaska.....	Long	320	196	39
Perfection....	Medium long	172	30	0
Hundredfold...	Medium long	124	66	30
Stratagem.....	Short	250	120	71
Little Marvel...	Short	163	50	21
Daisy.....	Very short, stubby	163	92	28

them all, but Alaska, Kelvedon Wonder, Little Marvel, Charles I, and Groene Lente were among the best. In view of these results it is surprising that JOST and REISS were not able to obtain satisfactory auxin curvatures with peas, since practically all varieties are good. In table 8 the curvatures of several pea varieties are listed. It is evident that the type of growth does not determine the suitability of the variety for the pea test.

## RELATION BETWEEN TIME AND CURVATURE

It has been shown for *Avena* coleoptiles that the rate of growth (curvature) is proportional to the concentration of the auxin applied. A difference between a low and a high concentration can be found even after a time so short that the curvatures are just beginning to develop. In the pea test, however, the rate of curvature is

the same at all concentrations, as is shown in figure 8. When the auxin concentration is low the curvature proceeds for a short time only; in high concentrations the curvature continues for a much longer period.

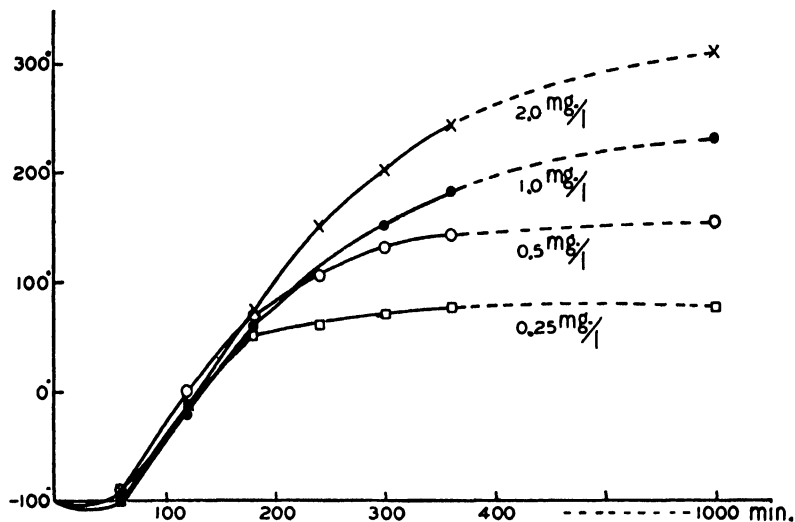


FIG. 8.—Progress of pea curvatures from moment that they are placed in indole-3-acetic acid solutions of different strength, until final curvature is reached, after 1000 minutes. Each curve represents curvature in degrees (ordinate) of fourteen halves. Initial negative curvature due to tissue tension. Rate of curvature 1-3 hours after placing stems in solution is constant, independent of final curvature.

### Summary

1. If growing stems of pea seedlings are split and immersed in a solution containing auxin, the two halves will curve inward (fig. 5). This pea test curvature is proportional to the logarithm of the auxin concentration (fig. 6). The curvature is due to differential growth.

2. The epidermis side of the halved stems grows faster than the wounded inside (fig. 1). This difference in growth in turn is caused by the fact that auxin is unable to enter the stem through the wounded surface. If auxin is forced into the halved stems by infiltration with auxin solutions, the inside will grow just as much as the unwounded epidermis side (table 6).

3. The pea test can be successfully used for quantitative auxin determinations, the technique of which is described.

4. The pea test curvature is dependent upon the pH of the auxin solutions (fig. 6). At greater acidities greater curvatures are obtained. As in the *Avena* coleoptile, the sensitivity of the pea stems to auxin has a daily and seasonal variation. A wide variety of pea races was found to be suitable material for the pea test. Of the fifty-five varieties investigated only two did not give any curvature at all. The rate of curvature was shown to be independent of the auxin concentration, but the higher the concentration the longer the period of time during which the curvature proceeds.

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# CHEMICAL STUDY OF THE RIPENING PROCESS OF BOSC PEARS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 481

WILLIAM E. MARTIN

(WITH FIVE FIGURES)

## Introduction

In the commercial production and marketing of pears the fruit is not allowed to ripen on the tree, but is picked while it is still hard and green. Winter varieties are usually placed in storage immediately after picking and packing, and are not marketed until later in the season. Some varieties ripen slowly at storage temperatures while others remain hard and green for long periods and do not ripen until removed from storage and kept for a time at a higher temperature. The Bosc pear is one of the latter class, and tests have shown (9) that ripening temperatures of  $65^{\circ}$ – $70^{\circ}$  F. are essential for the development of best eating quality of the fruit.

The process of ripening or conditioning the fruit at these latter temperatures has become general in recent years, and much of the fruit of this variety is brought to the condition known as "firm eating ripe" before it is offered on the retail market.

The physiology of the ripening of summer pears such as the Bartlett has been investigated by several workers (1, 6, 12). There has been comparatively little work done on the chemical changes involved in the stages of ripening of winter pears taking place before the fruit is placed on the market, and no work at all, to the writer's knowledge, on the changes taking place after the fruit has attained optimum eating quality.

In the present investigation the attempt was made to determine chemically the nature of the metabolic changes taking place during the ripening of Bosc pears at  $67^{\circ}$  F. after removal from storage. The experiments were planned to determine the nature of the ripening process after the point of optimum eating quality had been reached and during the time the fruit was becoming overripe and developing

“internal breakdown.” This breakdown, known also as core rot, is characterized by a brown discoloration and softening of the tissues about the core. It appears first at the stem end of the fruit along the vascular elements leading to the core and later develops in the pith parenchyma surrounding the carpels. HARLEY and FISHER (7) have demonstrated that this breakdown of the pear is associated with an accumulation of acetaldehyde in the tissues, and have suggested that after a certain concentration of acetaldehyde is reached browning and breakdown take place.

Bosc pears taste very much sweeter at the time of optimum eating quality than they do when either green or overripe. It was planned, therefore, to determine accurately whether there was any appreciable increase in sugars during ripening or whether the observed increase in sweetness was merely caused by a concentration of solutes as the fruit lost water by transpiration.

The experiment was carried out with three different lots of fruit, one picked at the time of commercial harvest, another ten days earlier, and the third ten days after the commercial crop. A number of representative samples which had received the same treatment as the commercial crop were placed in a ripening room at 67° F., and samples were withdrawn for analysis on each of twenty successive days. At the time the fruit was placed in the ripening room the pears of all three pickings were still hard. The green ground color was beginning to fade and patches of yellow were appearing in the fruit of the late picking.

### Material and methods

The fruit used in this investigation was obtained through the courtesy of F. C. REIMER of the Southern Oregon Experiment Station, Talent, Oregon, and was picked from a single Bosc tree.

Three pickings of four boxes each were made on September 3, 13, and 23, 1935. The picking on September 13 corresponded with the commercial harvest of Bosc pears in the region; the other pickings were respectively earlier and later. The maturity of the fruits was measured by the Oregon pressure tester (13) and the results of these measurements are listed in table 1. The pressure test is based on the fact that during the growth and ripening of the pear there is a

gradual and consistent lowering of the physical resistance to pressure or wounding of the epidermal and cortical regions of the fruit.

Immediately after picking, the fruit was wrapped and packed in boxes and placed in storage at  $30^{\circ}$ – $31^{\circ}$  F., and on November 16 it was removed from storage and shipped to Chicago under ventilation, arriving November 25. After arrival, the fruit was removed immediately from the shipping boxes and graded on the basis of size. All fruits injured by stem punctures or damaged by insects were discarded.

The fruit of each picking was then divided into twenty representative lots containing an equal number of large, medium, and small pears. In series A there were twenty pears in each of the twenty

TABLE 1  
MATURITY OF PEARS AT TIME OF PICKING

SERIES	PICKED	PRESSURE IN POUNDS
A . . . . .	September 3	24 8
B . . . . .	September 13	21.3
C . . . . .	September 23	17 9

sample lots put up for ripening. The fruits of the second and third pickings (series B and C) were somewhat larger and only eighteen representative pears were taken for each sample lot. Thus the fruit of each picking was divided into twenty representative sample lots of eighteen or twenty pears each. All of the sixty samples thus prepared were weighed and placed in a ripening room in which the temperature was maintained at  $67^{\circ}$  F.

Three sample lots, one of each series, were removed from the ripening room on each of 20 successive days. Thus three series of samples were obtained and each series represented all stages of ripeness, ranging from the green condition to fruit of good eating quality and continuing on to overripeness and "internal breakdown" owing to natural causes. It was planned to obtain samples which would yield data, not only on the sequence of chemical events involved in ripening, but also on the effects of time of picking upon the ripening process.

It was found necessary to modify the experiment somewhat, since

it was desirable to discard all sample lots which had decayed during the ripening period. Daily samples were taken for 17-20 days, depending upon the number of undecayed sample lots available in each case.

#### METHODS OF ANALYSIS

At the time of removal from the ripening room the fruit was again weighed carefully and the loss of weight during the period of ripening computed for each of the sample lots examined.

Following weighing, samples of each lot were taken for analysis. This was accomplished by cutting two opposite longitudinal sectors from each pear, grinding the sectors through a Russwin food chopper, and then weighing duplicate 50 gm. samples from the ground pulp. These samples were preserved by boiling with sufficient 95 per cent ethyl alcohol to obtain a final concentration of 80 to 85 per cent. The preserved samples were then set aside for chemical analysis.

At the time samples were taken for analysis an attempt was made to gauge roughly the eating quality of the fruit. While such tests were obviously inaccurate, approximate data were obtained as to the time in the sequence of sampling at which the fruit was of good eating quality.

**EXTRACTION.**—This was carried out with the Soxhlet apparatus. The preserved samples were transferred to a weighed filter paper folded into a paper extraction cup and the filtrate collected in a volumetric flask. The extraction cup and contents were then placed in the Soxhlet apparatus and extracted with 95 per cent ethyl alcohol for at least 24 hours and until the liquid in the upper receiver showed a negative alpha-naphthol carbohydrate test. The extract was then added to the original alcoholic filtrate and the sample made up to 500 ml. with 95 per cent ethyl alcohol. The solution thus prepared contained the alcohol soluble solids.

**ALCOHOL SOLUBLE SOLIDS.**—These were determined gravimetrically by pipetting an aliquot of the alcohol extract into a tared 100 ml. beaker, evaporating the alcohol on the steam bath, and finally drying in a vacuum oven at 75° C. After 65 hours' drying approximately constant weight had been reached and the residue was then weighed.



**ALCOHOL INSOLUBLE RESIDUE.**—The residue was determined similarly by drying the tared filter paper and contents after removal from the Soxhlet apparatus. The initial weight of the filter paper and the final weight of the filter paper and residue were both obtained by drying to constant weight at 75° C. in the vacuum oven. All weighings were made in large weighing bottles to prevent absorption of moisture by the dry filter papers and residues.

**TOTAL SOLIDS.**—These were calculated as the sum of the alcohol soluble solids and the alcohol insoluble residue.

**SUGAR DETERMINATIONS.**—Determinations were made in such a way as to yield the relative amounts of sucrose, dextrose, and levulose present in the pear. Aliquot fractions of the alcohol extracts were pipetted into 250 ml. volumetric flasks and the alcohol evaporated on the steam bath. This was readily accomplished by inserting glass tubes into the necks of the flasks and blowing a stream of air on the liquid while heat was applied from below. The alcohol evaporated readily and was replaced by two additions of distilled water, 25 ml. each, after which the volume was finally reduced to about 10 ml.

The aqueous extract thus obtained was cleared by the addition of 5 ml. of a saturated solution of neutral lead acetate, delead with a slight excess of potassium oxalate, made up to volume, and filtered at once into a dry flask. The clear solution thus obtained was then ready for the determination of reducing sugars before inversion.

A separate solution was prepared for the determination of reducing sugars after inversion. The procedure was the same as the preceding, except that the aqueous extract before clarification was made up to about 50 ml., acidified with five drops of 10 per cent acetic acid and inverted by five drops of a 1 per cent solution of invertase scales. The reaction mixture was allowed to stand overnight at room temperature and then cleared, delead, and filtered as described.

**REDUCING SUGARS BEFORE AND AFTER INVERSION.**—These were determined by a modification of the Official Methods (2). Reduction was carried out according to the conditions of Munson and Walker, and the  $\text{Cu}_2\text{O}$  collected on asbestos pads in Gooch crucibles. The pad and  $\text{Cu}_2\text{O}$  were then suspended in 25 ml. of hot water and

the  $\text{Cu}_2\text{O}$  dissolved by heating after adding 5 ml. of 50 per cent sulphuric acid and a slight excess of a saturated solution of potassium permanganate. The excess permanganate was then decomposed by adding a saturated solution of potassium oxalate dropwise until the color of the permanganate disappeared and the pale blue of the cupric sulphate remained. The excess of the oxalate solution was limited to one drop. After cooling, 10 ml. of a 30 per cent solution of potassium iodide was added and the liberated iodine titrated with 0.05 N sodium thiosulphate. One ml. of 0.05 N sodium thiosulphate is equivalent to 3.597 mg. of  $\text{Cu}_2\text{O}$ . The sugar equivalent to the  $\text{Cu}_2\text{O}$  was obtained from the Munson and Walker tables and was expressed both as invert sugar and as dextrose.

**SUCROSE.**—This was calculated from the difference in reducing sugars before and after inversion, and is expressed as sucrose.

**DEXTROSE AND LEVULOSE.**—These were determined by the method of LOTHROP and HOLMES (11), and the determinations were carried out on samples drawn from the same solutions that were prepared for the determination of reducing sugars before inversion.

**TOTAL SUGARS.**—These were calculated as the numerical sum of the determined amounts of sucrose, dextrose, and levulose.

**NON-SUGAR SOLUBLE SOLIDS.**—These were calculated as the difference between the alcohol soluble solids and the total sugars.

**SORBITOL.**—This was determined by a modification of WERDER's method (18). This type of method depends on the formation of dibenzal-sorbitol from sorbitol and benzaldehyde in the presence of strong sulphuric acid as a condensing agent. In order to obtain quantitative yields of dibenzal-sorbitol the proportions of sorbitol, benzaldehyde, and sulphuric acid must be carefully controlled. Similarly the time and temperature of condensation and the method of treatment of the precipitated dibenzal-sorbitol must be judiciously carried out if approximately theoretical yields are to be obtained.

Several quantitative methods were tried out using pure sorbitol (Pfanstiehl). The method of BLEYER, DIEMAIR, and LIX (3) was found most satisfactory. Approximately quantitative yields of dibenzal-sorbitol were obtained if the precaution was taken of neutralizing the sulphuric acid with a slight excess of sodium carbonate, bringing to a boil, and finally cooling before filtering off the dibenzal,

drying and weighing. It was found that the dibenzal-sorbitol so treated could be dried at  $100^{\circ}$  C. without decomposition and that after 10–12 hours a constant weight had been attained.

The general method and proportions of the reagents used in the determination of sorbitol were essentially those listed by BLEYER, DIEMAIR, and LIX in their modification of WERDER's original procedure, but certain further modifications were made and the procedure used in this investigation is briefly described.

About 75 ml. of the alcohol extract containing the soluble solids was pipetted into a flask and 1 gm. of Mallinkrodt activated charcoal was added. The flask was tightly corked, well shaken, and the solution filtered at once through a fluted filter paper (Whatman no. 42) into a dry flask. The filtration was rapid and within 10–15 minutes a 50 ml. aliquot of the clear filtrate was pipetted into a 100 ml. beaker and evaporated on the steam bath to a very thick syrup, which solidified on cooling.

One ml. of 50 per cent sulphuric acid (1:1) and 0.5 ml. of benzaldehyde were added and the mixture stirred well for about 5 minutes and then placed in the refrigerator ( $1^{\circ}$ – $3^{\circ}$  C.) for 19–20 hours. During this time a thick yellowish white paste was formed. Fifty ml. of cold distilled water was then added and the paste broken up with a stirring rod. After remaining in the refrigerator for two hours longer a slight excess of anhydrous sodium carbonate was added (1.5 gm.). Finally one hour later the material was removed from the refrigerator, boiled for one to two minutes, and cooled in running water. The white amorphous precipitate of dibenzal-sorbitol was then collected in a tared gooch crucible, washed thoroughly with distilled water, and weighed after drying 10–12 hours at  $100^{\circ}$  C. One hundred mg. of sorbitol is equivalent theoretically to 196.8 mg. of dibenzal-sorbitol.

This method was found to give approximately quantitative yields in the range between 60 and 250 mg. of sorbitol per determination. The results of chemical tests following this procedure are shown in table 2.

The necessity of removal of sugars before determining sorbitol has been stressed by several workers (3, 15), who have reported low yields of dibenzal-sorbitol if sugars were not removed. In this in-

TABLE 2  
QUANTITATIVE DETERMINATION OF SORBITOL

SORBITOL TAKEN	WEIGHT OF DIBENZAL-SORBITOL		PERCENTAGE YIELD
	OBTAINED	THEORETICAL	
75. . . . .	148.8	147.6	100.8
75. . . . .	149.9	147.6	100.6
100. . . . .	198.0	196.8	100.6
100. . . . .	200.0	196.8	101.6
125. . . . .	240.2	246.0	97.6
125. . . . .	240.0	246.0	97.6
150. . . . .	277.4	295.2	83.8
150. . . . .	267.4	295.2	90.6
175. . . . .	346.7	344.4	100.7
175. . . . .	344.7	344.4	100.1
200. . . . .	395.2	393.6	100.4
200. . . . .	398.9	393.6	101.3
250. . . . .	492.1	492.0	100.0
250. . . . .	492.1	492.0	99.8

TABLE 3  
EFFECTS OF SUGARS ON DETERMINATION OF SORBITOL

SORBITOL TAKEN (MG.)	SORBITOL FOUND			
	SORBITOL ALONE		SORBITOL AND SUGAR*	
	WEIGHT (MG.)	YIELD (%)	WEIGHT (MG.)	YIELD (%)
75. . . . .	78.7	104.4	77.6	103.2
100. . . . .	105.8	105.8	105.8	105.8
125. . . . .	134.8	107.8	131.0	105.0
150. . . . .	144.4	96.3	154.4	103.0
175. . . . .	168.0	96.0	171.3	97.8
200. . . . .	184.6	92.5	191.1	96.4

\* The same amount of dextrose, sucrose, and levulose was added to each sample as was present in a 50 ml. aliquot of the extract of sample B-6.

vestigation, however, it was found that if condensation were allowed to proceed for 19-20 hours in the refrigerator at  $1^{\circ}$ - $3^{\circ}$  C. approximately quantitative yields of the dibenzal derivative were obtained from the analysis of solutions of pure sorbitol to which definite amounts of sugar had been added. The results of such tests are shown in table 3. The amounts of dextrose, levulose, and sucrose added to each sample were approximately the same as had been determined to be present in 50 ml. aliquots of pear extracts.

It may be seen from table 3 that the method used in this investigation is approximately quantitative and that errors did not exceed 8 per cent of correct values. Tests with much larger amounts of dextrose added alone to pure sorbitol showed less effect on the determination of sorbitol than shown in table 3.

### Presentation of data

#### LOSS OF WEIGHT DURING RIPENING

The relative weight losses of pears of the three different pickings are shown in table 4 and in figure 1. These losses are expressed as percentages of the original weight of the fruit at the time it was placed in the ripening room, and represent the sum of the water loss, the carbon dioxide loss, and the loss due to other volatile products evolved by the fruit during metabolism.

It may be noted that the fruit of series B, picked September 13, lost weight somewhat more rapidly than did the fruit of series A and C which were picked on September 3 and 23 respectively. The magnitude of the weight loss was about 0.5 per cent per day in the fruit of series A and C, which corresponds approximately to the weight losses reported by HARTMAN (8) in his investigation of Bartlett pears held at  $66^{\circ}$  F. In over 400 hours there was no apparent reduction of the rate of weight loss, except perhaps in series B where a larger percentage of the original weight had been lost.

#### COMPOSITION OF PEARS AT VARIOUS STAGES OF RIPENING

The data presented in table 5 show the changes in the concentration of sugars and other constituents of the fruit of the three pickings as measured by the composition of individual sample lots

taken at daily intervals during the ripening period. These data are expressed as percentages of the fresh weight of the fruit at the time of withdrawal from the ripening room and thus give a measure of the composition of the fruit in so far as determined in this investigation.

TABLE 4

EFFECT OF TIME OF PICKING ON LOSS OF WEIGHT DURING RIPENING

SERIES A SEPTEMBER 3, 1935			SERIES B SEPTEMBER 13, 1935			SERIES C SEPTEMBER 23, 1935		
HOURS RIPENED	LOSS OF WEIGHT (%)	PART OF ORIGINAL WEIGHT*	HOURS RIPENED	LOSS OF WEIGHT (%)	PART OF ORIGINAL WEIGHT*	HOURS RIPENED	LOSS OF WEIGHT (%)	PART OF ORIGINAL WEIGHT*
13.....	0.36	0.9964	22	0.70	0.9924	25	0.65	0.9935
37.....	0.96	0.9904	46	1.67	0.9833	48	1.29	0.9871
61.....	1.39	0.9861	62	2.05	0.9795	70	1.36	0.9864
81.....	2.09	0.9791	94	2.95	0.9795	95	1.75	0.9825
118.....	2.86	0.9714	121	3.54	0.9646	122	2.78	0.9722
141.....	3.52	0.9648	143	4.32	0.9568	145	2.90	0.9710
166.....	3.96	0.9604	166	5.08	0.9492	169	3.24	0.9676
192.....	4.35	0.9565	192	5.91	0.9409	191	4.24	0.9576
214.....	5.05	0.9495	213	7.11	0.9289	213	4.82	0.9518
234.....	4.94	0.9506	237	7.84	0.9216	235	4.73	0.9627
260.....	5.72	0.9428	263	8.33	0.9167	265	5.39	0.9461
285.....	6.38	0.9362	286	8.18	0.9182	287	5.55	0.9445
308.....	7.04	0.9296	309	8.59	0.9141	309	6.85	0.9315
325.....	7.15	0.9285	331	9.48	0.9052	333	7.44	0.9256
360.....	7.83	0.9217	360	10.09	0.8991	361	7.84	0.9216
384.....	8.46	0.9154	384	11.81	0.8819	408	.....	.....
405.....	.....	.....	404	11.02	0.8894	457	9.89	0.9011
432.....	10.10	0.8990	.....	.....	.....	.....	.....	.....
480.....	10.83	0.8917	.....	.....	.....	.....	.....	.....

\* Part or proportion of original weight remaining.

The data obtained from the three different sets of fruit, picked ten days apart, show very similar composition and exhibit the same trends during the ripening process. Owing to this similarity only the data of series B are presented (fig. 2).

It may be seen from figure 2 that there was a definite increase in the total sugar content of the fruit as it ripened and that this increase continued throughout the whole period observed. The concentration of sucrose increased for the first six days of ripening and then decreased rapidly and continually for the remainder of the

period. The concentration of dextrose remained approximately constant until the decrease in sucrose was observed, and then increased as the amount of sucrose became less. Levulose increased in concentration steadily during the whole of the ripening process.

Fruit of good eating quality was obtained between the sixth and tenth day. Thereafter all fruit was inferior and internal breakdown became increasingly widespread from the eleventh day to the end of the period.

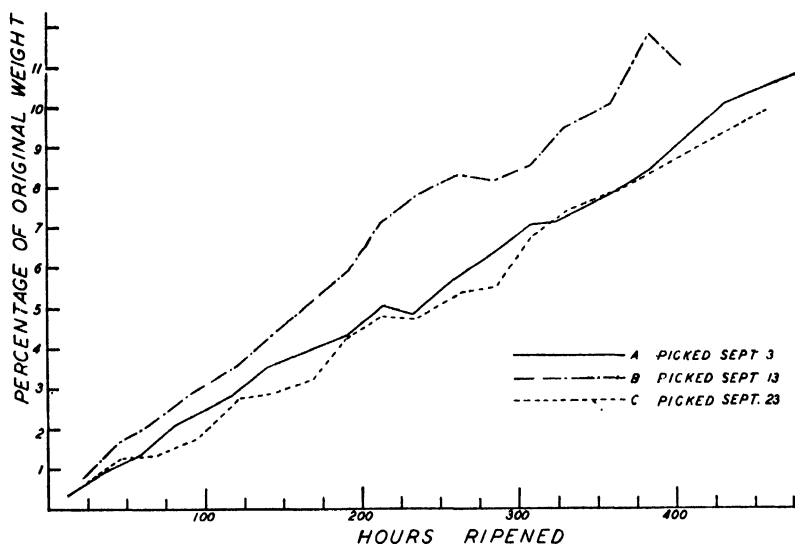


FIG. 1.—Weight loss during ripening of Bosc pears.

There were considerable fluctuations in the observed values of total solids and these fluctuations caused a marked scatter in the points upon the total solids curve as shown in figure 2.

The interpretation of data expressed in terms of fresh weight at the time of sampling may lead to erroneous conclusions, as will be shown later. The data shown in figure 2 and table 5 are presented, however, since they represent a measure of the actual composition, and since most of the American work on the physiology of fruit ripening has been expressed on this basis.

TABLE 5  
COMPOSITION OF BOSCH PEARS EXPRESSED AS PERCENTAGE  
FRESH WEIGHT AT TIME OF SAMPLING

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series A, picked September 3, 1935									
1	1.95	5.47	1.93	9.35	4.54	3.73	13.89	4.30	18.19
2	1.64	5.76	2.38	9.78	4.31	3.54	14.09	4.04	18.13
3	1.95	5.47	2.08	9.50	3.99	3.18	13.49	3.94	17.43
4	1.99	5.74	2.67	10.40	4.26	3.08	14.66	4.13	18.79
5	1.76	6.06	2.62	10.44	3.46	2.87	13.90	3.81	17.71
6	1.70	6.05	2.60	10.35	3.25	2.61	13.60	3.62	17.22
7	2.09	6.16	2.02	10.27	3.54	2.56	13.81	3.67	17.48
8	2.20	6.11	1.99	10.30	3.36	2.39	13.66	3.87	17.53
9	2.29	6.61	2.29	11.19	2.96	2.33	14.15	3.90	18.05
10	2.30	6.69	2.19	11.18	2.81	2.23	13.99	3.68	17.67
11	.....	.....	1.62	.....	.....	2.17	13.53	3.41	16.94
12	2.40	6.71	1.48	10.59	2.59	2.09	13.18	3.77	16.95
13	2.57	6.88	1.08	10.53	2.78	.....	13.31	3.56	16.87
14	2.63	7.36	1.00	10.99	3.06	1.98	14.05	3.52	17.57
15	2.72	7.31	.86	10.89	2.90	.....	13.79	3.79	17.58
16	2.79	7.16	.81	10.76	2.93	1.89	13.69	3.69	17.38
17	2.86	7.54	.81	11.21	2.97	.....	14.18	3.74	17.92
18	2.97	7.76	.71	11.44	3.00	1.98	14.44	3.96	18.40
20	2.93	7.56	.66	11.15	2.87	1.93	14.02	3.88	17.90
Series B, picked September 13, 1935									
1	1.68	5.06	2.94	9.68	4.23	3.31	13.91	3.31	17.56
2	1.67	5.25	3.12	10.04	4.25	3.24	14.29	3.79	18.08
3	1.69	5.08	3.51	10.28	3.80	2.99	14.08	3.84	17.92
4	1.68	5.56	3.70	10.94	3.82	2.88	14.76	3.71	18.47
5	1.66	5.59	3.79	11.04	3.77	2.71	14.81	3.60	18.41
6	1.71	5.76	3.90	11.37	3.29	2.59	14.66	3.89	18.55
7	1.85	5.90	3.77	11.52	3.31	2.32	14.83	3.77	18.60
8	1.88	6.22	3.37	11.47	3.20	2.11	14.67	3.73	18.40
9	1.92	6.38	3.68	11.98	3.14	2.21	15.12	3.63	18.75
10	2.11	6.45	2.94	11.50	3.17	2.15	14.67	3.88	18.55
11	2.24	6.88	3.18	12.30	3.23	2.17	15.53	3.67	19.20
12	2.57	6.97	2.54	12.08	3.10	2.18	15.18	3.90	19.08
13	2.60	6.98	2.22	11.80	2.99	1.94	14.79	3.81	18.60
14	2.75	7.04	2.00	11.79	2.88	1.95	14.67	3.86	18.53
15	2.96	7.12	1.90	11.98	3.10	1.97	15.08	3.97	19.05
16	3.04	7.65	1.71	12.40	2.99	1.98	15.39	3.91	19.30
17	3.28	7.72	1.68	12.68	2.90	.....	15.58	3.68	19.26



TABLE 5—*Continued*

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series C, picked September 23, 1935									
3.....	1.60	5.26	4.15	11.01	3.98	2.90	14.99	3.41	18.40
4.....	1.48	5.49	4.47	11.44	4.03	3.07	15.47	3.36	18.83
5.....	1.54	5.46	4.31	11.31	3.53	2.58	14.84	3.37	18.21
6.....	1.55	5.78	4.37	11.70	3.52	2.41	15.22	3.35	18.57
7.....	1.55	5.89	4.74	12.18	3.51	2.46	15.69	3.42	19.11
8.....	1.76	6.17	4.13	12.06	3.36	2.12	15.42	3.31	18.73
9.....	1.89	6.41	4.10	12.40	3.42	2.19	15.82	3.28	19.10
10.....	2.03	6.58	3.72	12.33	3.37	.....	15.70	3.45	19.15
11.....	2.33	6.63	3.21	12.17	3.45	2.08	15.62	3.22	18.84
12.....	2.49	7.03	2.70	12.22	3.12	2.05	15.34	3.24	18.58
13.....	2.80	6.80	2.94	12.54	2.80	1.90	15.34	3.35	18.69
14.....	2.84	7.48	2.62	12.94	2.98	1.91	15.92	3.36	19.28
15.....	2.91	7.45	2.20	12.56	2.93	1.93	15.49	3.31	18.80
17.....	3.23	7.94	1.39	12.56	2.60	1.94	15.16	3.40	18.56
19.....	3.43	8.00	1.23	12.66	2.80	1.85	15.46	3.57	19.03

## CHEMICAL CHANGES TAKING PLACE DURING RIPENING

The results presented in the preceding section were listed as percentages of the fresh weight at the time the samples were removed from the ripening room. In a quantitative study of chemical changes in apples, HAYNES and ARCHBOLD (10) have shown that results expressed in terms of fresh weight at the time of sampling do not necessarily show the actual changes in the various constituents of the fruit, and that, if a correct picture of the metabolic process is to be obtained, results should be expressed in terms of original fresh weight.

It was shown earlier in this paper that marked losses of weight took place as the fruit ripened. These losses amounted to about 0.5 per cent of the original fresh weight per day and were probably largely water. It might be expected that the concentration of solutes in the cell sap would increase as evaporation of water took place. By expressing results as percentages of fresh weight an increase in sugars might be observed, although no actual synthesis had taken place and the sugar already present merely had come to represent an increasing proportion of the fresh weight of the fruit.

In series B, in which the amounts of total sugars definitely increased when expressed as percentages of fresh weight at the time

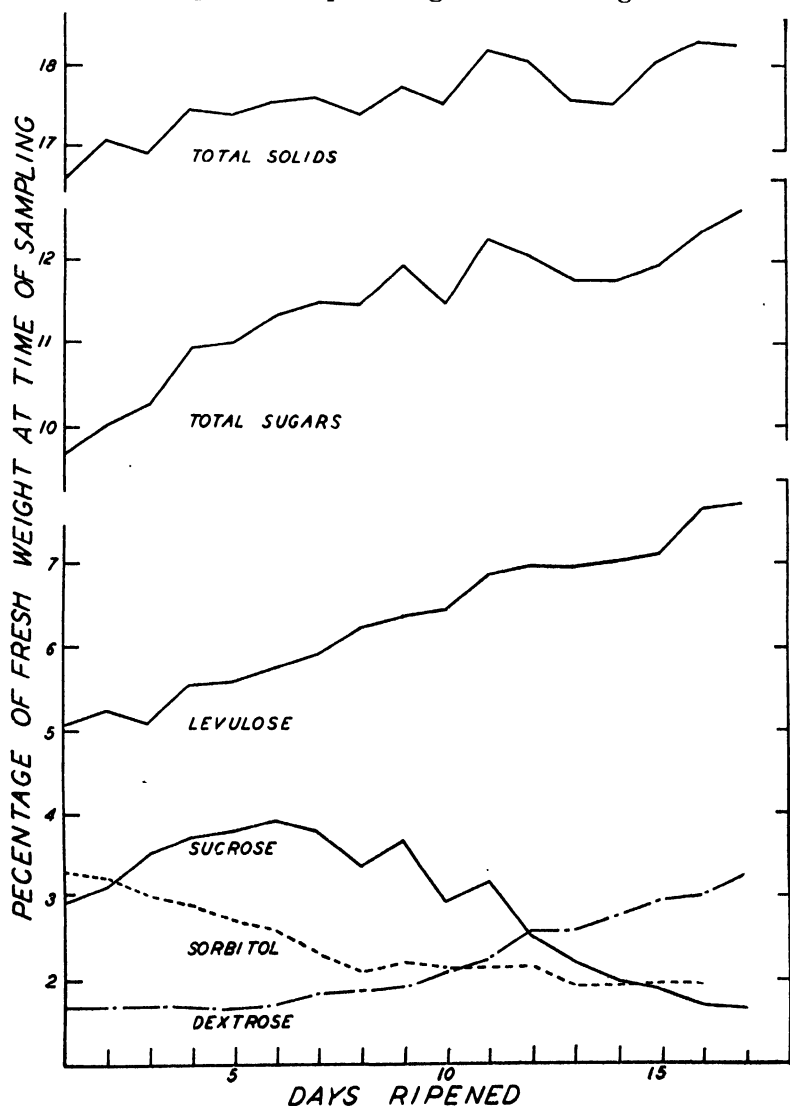


FIG. 2.—Changes in composition of Bosc pears during ripening.

of sampling, an apparent increase in total solids was also observed, as may be seen from figure 2. It seems unlikely, however, that the

amount of solid material in a pear should increase appreciably during ripening, especially in view of the fact that there should be decreases in total solids owing to losses of carbon dioxide and other volatile products of the metabolism of the fruit.

This apparent discrepancy may be explained if we recall that 11 per cent of the weight of the fruit was lost, leaving only 89 per cent of the original weight of the sample after seventeen days of ripening. Thus a 50 gm. sample taken after seventeen days represented a considerably larger proportion of the fruit than did a 50 gm. sample at the beginning of the tests. If a 100 gm. fruit were analyzed, the 50 gm. sample taken at the start would represent one half of the fruit, but after seventeen days would represent  $\frac{59}{89}$  of the fruit. If results were expressed only in terms of fresh weight a comparison might be made between 100 gm. of original fruit and tissue equivalent to 112 gm. when analysis was made seventeen days later. To correct for this error in the effective size of sample, the results may be multiplied by 0.89, which is the value of the proportion of original fresh weight remaining after seventeen days of ripening. After carrying out this calculation it is found that total solids at the start of ripening represented 17.43 per cent of the original fresh weight, while after seventeen days the total solids were only 17.14 per cent of the original fresh weight of the fruit.

Thus there was actually a slight decrease in the total solid material during ripening, whereas the calculation of results in terms of fresh weight at time of sampling showed an increase in total solids.

In order to ascertain the actual changes in the various constituents of the fruit as ripening took place, the data shown in table 5 were recalculated as percentages of the original fresh weight. This was accomplished by multiplying the values listed in the table by the corresponding values of "proportion of original weight remaining" which are listed in table 4. By these calculations all data were reduced to a comparable basis, percentages of original fresh weight, and have been so listed in table 6. As before, only the data of series B are presented (fig. 3), since the changes shown by the fruit of this series appear to be representative of all three sets studied.

**CARBOHYDRATE CHANGES IN RIPENING.**—The data plotted in figure 3 show a definite increase in levulose throughout the whole

TABLE 6  
CHANGES IN CONSTITUENTS OF BOSCH PEARS EXPRESSED AS  
PERCENTAGE ORIGINAL FRESH WEIGHT

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	TOTAL SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series A, picked September 3, 1935									
1	1.04	5.45	1.93	9.32	4.52	3.72	13.84	4.28	18.12
2	1.62	5.70	2.36	9.68	4.27	3.51	13.95	4.00	17.95
3	1.92	5.39	2.06	9.37	3.93	3.14	13.30	3.88	17.18
4	1.95	5.62	2.61	10.18	4.17	3.02	14.35	4.05	18.40
5	1.71	5.88	2.55	10.14	3.36	2.79	13.50	3.70	17.20
6	1.64	5.84	2.51	9.99	3.13	2.52	13.12	3.49	16.61
7	2.01	5.92	1.94	9.87	3.40	2.46	13.27	3.52	16.79
8	2.10	5.85	1.90	9.85	3.22	2.29	13.07	3.70	16.77
9	2.17	6.28	2.17	10.62	2.81	2.21	13.43	3.70	17.13
10	2.19	6.36	2.08	10.63	2.67	2.12	13.30	3.50	16.80
11			1.53			2.05	12.76	3.21	15.97
12	2.25	6.28	1.39	9.92	2.42	1.96	12.34	3.53	15.87
13	2.40	6.40	1.00	9.80	2.58		12.38	3.30	15.68
14	2.44	6.83	.93	10.20	2.84	1.84	13.04	3.27	16.31
15	2.51	6.74	.79	10.04	2.67		12.71	3.49	16.20
16	2.55	6.55	.75	9.85	2.68	1.73	12.53	3.38	15.91
18	2.67	6.98	.64	10.29	2.70	1.78	12.90	3.55	16.54
20	2.61	6.74	.59	9.94	2.56	1.72	12.50	3.46	15.96
Series B, picked September 13, 1935									
1	1.67	5.02	2.92	9.61	4.20	3.29	13.80	3.62	17.42
2	1.64	5.16	3.07	9.87	4.18	3.19	14.05	3.73	17.78
3	1.65	4.98	3.44	10.07	3.72	2.93	13.79	3.76	17.55
4	1.63	5.40	3.59	10.62	3.71	2.80	14.33	3.60	17.93
5	1.60	5.39	3.66	10.65	3.64	2.61	14.29	3.47	17.76
6	1.64	5.51	3.73	10.88	3.15	2.48	14.03	3.72	17.75
7	1.76	5.60	3.58	10.94	3.14	2.20	14.08	3.58	17.66
8	1.77	5.85	3.17	10.79	3.01	1.98	13.80	3.51	17.31
9	1.78	5.93	3.42	11.13	2.92	2.05	14.05	3.37	17.42
10	1.94	5.94	2.72	10.60	2.92	1.98	13.52	3.58	17.10
11	2.05	6.31	2.92	11.28	2.96	1.99	14.24	3.36	17.60
12	2.36	6.40	2.33	11.09	2.85	2.00	13.94	3.58	17.52
13	2.38	6.38	2.03	10.79	2.73	1.77	13.52	3.48	17.00
14	2.49	6.37	1.81	10.67	2.61	1.77	13.28	3.49	16.77
15	2.66	6.40	1.71	10.77	2.79	1.77	13.56	3.57	17.13
16	2.68	6.74	1.51	10.93	2.64	1.75	13.57	3.45	17.02
17	2.92	6.87	1.49	11.28	2.58		13.86	3.27	17.13

TABLE 6—*Continued*

DAYS RIPENED	DEX- TROSE	LEVU- LOSE	SU- CROSE	TOTAL SUGARS	NON- SUGAR SOLUBLE SOLIDS	SORBI- TOL	TOTAL SOLUBLE SOLIDS	ALCOHOL INS. RES.	TOTAL SOLIDS
Series C, picked September 23, 1935									
3	1.58	5.19	4.09	10.86	3.93	2.86	14.79	3.36	18.15
4	1.46	5.39	4.39	11.24	3.96	3.02	15.20	3.30	18.50
5	1.50	5.31	4.19	11.00	3.43	2.51	14.43	3.28	17.70
6	1.51	5.61	4.24	11.36	3.42	2.34	14.78	3.25	18.03
7	1.50	5.70	4.59	11.79	3.40	2.38	15.19	3.30	18.49
8	1.69	5.91	3.95	11.55	3.22	2.03	14.77	3.17	17.94
9	1.80	6.10	3.90	11.80	3.26	2.08	15.06	3.12	18.18
10	1.93	6.27	3.54	11.74	3.21	.....	14.95	3.29	18.24
11	2.20	6.27	3.04	11.51	3.26	1.97	14.77	3.05	17.82
12	2.35	6.64	2.55	11.54	2.95	1.94	14.49	3.06	17.55
13	2.61	6.33	2.74	11.68	2.61	1.77	14.29	3.12	17.41
14	2.63	6.92	2.43	11.98	2.76	1.77	14.74	3.11	17.85
15	2.68	6.87	2.03	11.58	2.70	1.78	14.28	3.05	17.33
19	3.09	7.21	1.11	11.31	2.52	1.67	13.93	3.22	17.15

of the ripening process. Sucrose increased during the first six days and then decreased steadily for the remainder of the period. While sucrose was increasing, dextrose remained constant; but as sucrose was hydrolyzed, dextrose appeared to be formed. As might be expected, the increases in dextrose and levulose accompanying the decrease in sucrose were approximately equal.

The points on the graphs show considerable scatter and it is difficult to judge the exact slope of the curves in some cases. The general trends may be observed, however, and a fair approximation made of the magnitude of the changes in the several sugars present.

Total sugars were found to increase definitely for the first six days; beyond that point little change could be observed with certainty owing to the scatter of the points on the curve. Total solids decreased during the ripening process. As has already been pointed out, this finding is more understandable than that observed when results were calculated as percentages of the fresh weight at the time of sampling.

The data for series A and C, the early and late picked fruit respectively, show essentially the same results as have been described,

except that the increases in total sugars were less marked and the decreases in total solids somewhat greater.

The early picked fruit contained less sucrose at all stages of ripe-

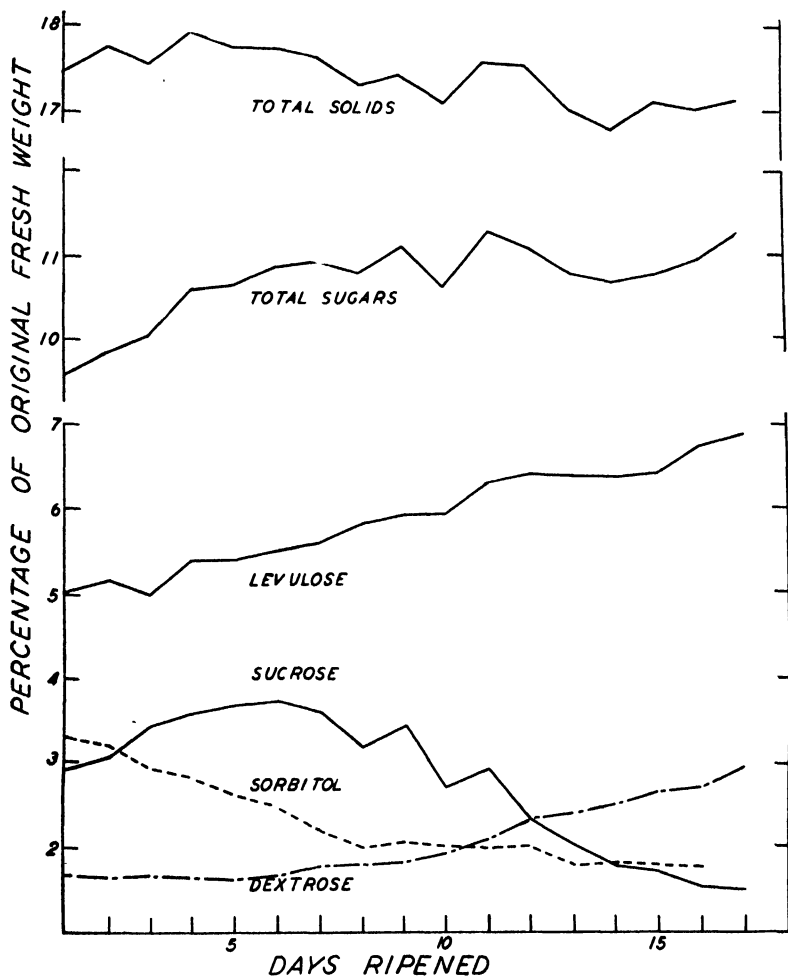


FIG. 3.—Changes which take place in the constituents of Bosc pears during ripening.

ness than did the fruit of the commercial picking. On the other hand, the late picked fruit showed more sucrose throughout the whole period than did the fruit picked at the time of commercial

harvest. A comparison of the relative sucrose content and its fluctuation during ripening is shown in figure 4. The dextrose and levulose contents of fruit of the three pickings were about the same and differences in total sugars in fruit of the three pickings were caused by the differences in sucrose.

Starch might be expected to serve as the material from which sugars were formed during the ripening process. Microchemical tests, however, indicated that only slight traces of starch were present. Moreover, the alcohol insoluble residue which would have con-

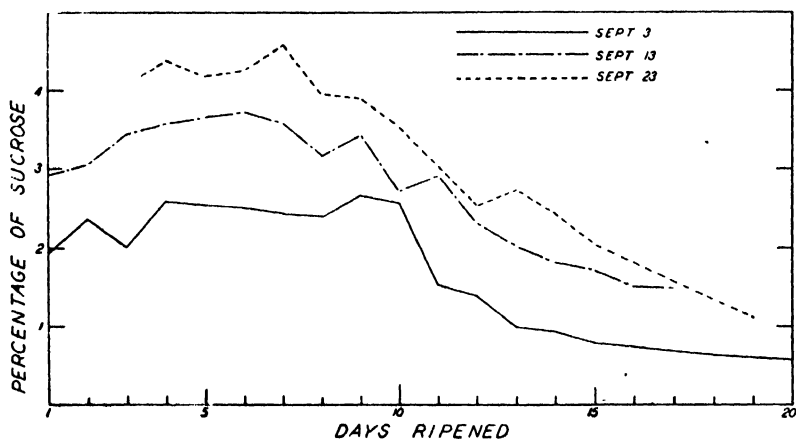


FIG. 4.—Effect of time of picking on sucrose changes during ripening.

tained the starch, if present, showed only very small decreases during the ripening of the fruit of series B and C, and only slight decreases in the fruit of series A. The magnitude of the observed decreases of this residue were found to be insufficient to account for the amounts of sugar observed to be formed during the ripening of the fruit. Starch determinations on the alcohol insoluble residues of the initial members of series A, B, and C showed 0.20, 0.20, and 0.28 per cent of starch respectively.

**SORBITOL CHANGES IN RIPENING.**—It was noticed from the data presented in the preceding section that the values of total sugars differed markedly from the corresponding values of soluble solids. This discrepancy was found to amount to 2.6–4.5 per cent of the fresh weight of the fruit, and suggested the presence of some major

constituent of a soluble non-sugar nature. The values of the differences between soluble solids and total sugars were calculated and designated non-sugar soluble solids. It was found that the values of this unknown undetermined constituent of the fruit decreased definitely during the ripening process and were roughly equal to or slightly in excess of the observed increases in total sugars. The values of this fraction of the fruit are shown in tables 5 and 6.

EMMETT (5) has reported that in the analysis of Conference pears there was a similar discrepancy between soluble solids and the sum of the separately determined constituents of the fruit. He calculated that 2 to 3 per cent of the fresh weight of the pear pulp was unidentified. Further, he noted that the amounts of this unidentified constituent decreased during storage, and suggested that the substance in question may have been sorbitol. He made no determinations, however, to substantiate this suggestion.

The presence of sorbitol in pears has been reported by VINCENT and DELACHANAL (17) and by REIF (15). Recently NUCCORINI and BARTOLI (14) have found that the sorbitol content of fruits of *Sorbus domestica* decreases when the fruit is detached from the tree, and they state that it is transformed into dextrose and levulose.

In view of these findings it was decided to determine qualitatively whether or not sorbitol occurred among the soluble solids of the Bosc pear. Certain of the alcohol extracts were evaporated to a thick syrup and this syrup allowed to react with benzaldehyde in the presence of sulphuric acid as a condensing agent. After standing overnight in the refrigerator, a white amorphous precipitate was obtained. This was then washed free of benzaldehyde and sulphuric acid. It was found to melt at  $174^{\circ}$ – $177^{\circ}$  C. and appeared to be dibenzal-sorbitol. DAVIS, SLATER, and SMITH (4) report that dibenzal-sorbitol prepared by this method melts at  $179^{\circ}$ – $184^{\circ}$  C. Owing to the difficulty of purifying this compound no recrystallization was carried out.

In order to demonstrate that the benzaldehyde derivative so obtained was definitely derived from sorbitol, a quantity of the material was hydrolyzed and then acetylated according to the methods given by TUTIN (16). The acetate so obtained was recrystallized from ethyl ether, and hard colorless crystals were ob-



tained. These crystals melted at  $98^{\circ}$ – $99^{\circ}$  C., which is the range reported by DAVIS, SLATER, and SMITH and by TUTIN for sorbitol hexaacetate. The acetate was also prepared by the same method from pure sorbitol; its melting point and appearance were found to be identical with that derived from pear extracts as just described.

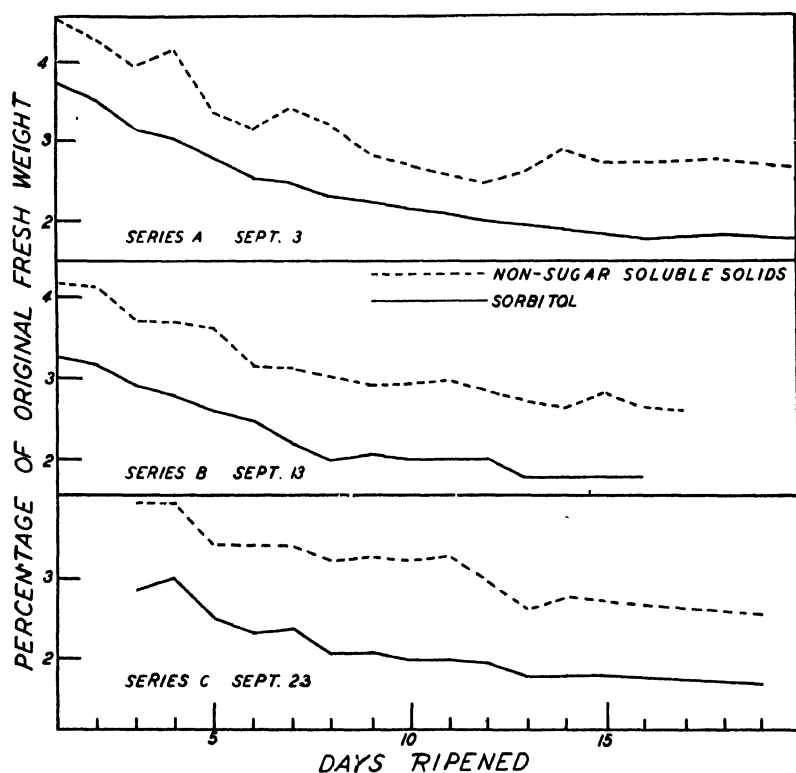


FIG. 5.—Relation of sorbitol to non-sugar soluble solids.

From the evidence presented it was assumed that sorbitol was definitely a constituent of the Bosc pear. Quantitative measurements were then made using the method outlined in the section on methods. The changes in sorbitol during ripening are listed in table 6 and shown graphically in figures 3 and 5. The changes in sorbitol (fig. 5) are plotted together with the corresponding changes in the

hitherto unidentified fraction which was designated as the non-sugar soluble solids.

The data shown in figure 5 indicate that sorbitol definitely decreases during the ripening process. In the fruit of all three pickings the decreases in the non-sugar soluble solids were accompanied by corresponding decreases in sorbitol. The most rapid decreases in sorbitol were found to take place in the first six to eight days, when both sucrose and levulose were observed to be formed from some hitherto unknown source.

The fact that the curves for sorbitol (fig. 5) follow the curves for non-sugar soluble solids indicates that the undetermined fraction contained sorbitol and that as sorbitol decreased the value of this fraction decreased. After subtracting sorbitol from the non-sugar soluble solids there still remained a value of 0.6 to 1.0 per cent, which seems reasonable for the sum of organic acids, soluble nitrogen, and other minor soluble constituents.

### Discussion

In previous reports on the chemical changes in the ripening of pears, the duration of ripening has been taken as the time required to obtain fruit of good eating quality. In this investigation the results include data on the period following the attainment of optimum eating quality and continue to the time of occurrence of severe internal breakdown. The period studied, according to the usual criteria, might be said to include both ripening and over-ripening. In comparing the data reported here with those of other published reports, therefore, comparable data must be taken.

The ripening process, from the point of view of sugar changes, may be considered to consist of two definite stages: the first in which sucrose is formed, and the second in which sucrose is hydrolyzed with corresponding increases in dextrose and levulose. The attainment of fruit of good eating quality appears to occur in the four days following the beginning of hydrolysis of sucrose.

The essential differences in the sugar content of ripe and over-ripe Bosc pears appear to be the relative amounts of sucrose and reducing sugars. Total sugars show no decrease with over-ripening, but the

amounts of reducing sugars are far greater and the amounts of sucrose far less than in fruit of good eating quality. The composition of sound and over-ripe Conference pears in storage has been determined by EMMETT (5), and his results show higher values of sucrose in sound than in over-ripe fruit, together with larger amounts of reducing sugars in over-ripe than in sound fruit. The results of the present investigation are in agreement with his.

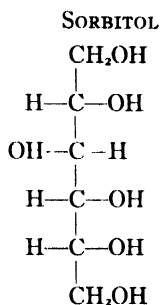
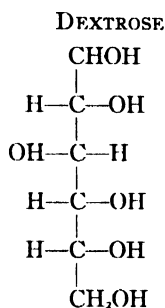
MAGNESS (12) and EZELL and DIEHL (6) have shown that in the ripening of Bartlett pears reducing sugars increased during ripening, and that in nearly all cases sucrose increased as well. If the composition of green Bosc pears is compared with that of ripe pears of good eating quality, essentially the same results may be observed, since during the first phase of ripening definite increases in the concentration of both sucrose and levulose were found.

MAGNESS has noted a much higher sugar content in fruit ripened at 70° F. than in comparable fruit ripened at lower temperatures. EMMETT (5), however, has suggested that the high sugar content observed by MAGNESS in fruit ripened at 70° F. may have been caused by a larger water loss by transpiration, for which no correction was made in the calculation of the sugar content. MAGNESS explained the larger increase in sugars at 70° F. as apparently due either to a smaller loss of sugar in respiration or to the fact that more insoluble or non-reducing substances in the green fruit are changed to soluble reducing material when the fruit is ripened at the optimum temperature.

The results presented in the preceding sections show the necessity of correcting for weight losses in ripening, but also indicate that even after such corrections are made, there are still definite increases in sugars which cannot be accounted for by decreases in the insoluble material of the fruit. The observed increases in sugars can easily be accounted for by the observed decreases in sorbitol. The presence of sorbitol in pears has been previously reported, but evidence for its transformation to sugars has not heretofore been presented.

Sorbitol may readily be oxidized to glucose chemically, and the manufacture of sorbitol is at present carried out by the reduction of glucose. It does not seem unlikely, therefore, that sorbitol could be oxidized to sugar in the processes of metabolism. The relationship

between the sugar alcohol, sorbitol, and dextrose may be seen from the following formulae of the two substances:



From the results presented here it appears that levulose and sucrose, rather than dextrose, are the sugars formed from sorbitol. During the most rapid decrease in sorbitol practically no dextrose was observed to be formed.

In the fruit of series B the transformation of sorbitol to sugars was almost quantitative, while in series A and C the decreases of sorbitol were considerably larger than the observed increases in sugars. In the latter two cases considerable losses in dry matter were noted, however, and it may be suggested that losses of sugar in respiration were sufficiently great that a smaller net gain in sugar was observed than would be expected from a quantitative oxidation of sorbitol to sugar.

NUCCORINI and BARTOLI have found that sorbitol is transformed to dextrose and levulose in the ripening of the fruits of *Sorbus domestica*. Such a transformation in the pear, accompanied by a preferential oxidation of dextrose in respiration, would lead to an excess of levulose over dextrose in the fruit. If the remaining dextrose combined with levulose to form sucrose and the excess levulose remained unchanged, we would observe in effect a transformation of sorbitol to sucrose and levulose, as appears to take place in the ripening of Bosc pears.

Whatever may be the mechanism of sorbitol metabolism in pears, it does definitely disappear in the ripening process. DAVIS, SLATER, and SMITH have found the heat of combustion of sorbitol in solution to be 732.44 kilogram calories per mol as compared with 676.08 for

dextrose. Thus an oxidation of a mol of sorbitol to glucose would release 66.36 kilogram calories of energy. The transformation of sorbitol to sugar by oxidation may therefore provide some of the energy for the metabolism of the fruit without loss of carbon dioxide, and thus represent a type of aerobic respiration which would not be recorded by the usual methods of measuring respiration.

### Summary

1. A study of the ripening process of Bosc pears held at 67° F. has been made by means of chemical analyses of individual sample lots removed from the ripening room on each of 17-20 successive days. Pears of three different lots, picked ten days apart, were studied in this way and determinations made of total solids, alcohol insoluble residue, soluble solids, dextrose, levulose, sucrose, and sorbitol.

2. The losses of weight during the ripening period were recorded. The results of sugar and other determinations were calculated in terms of fresh weight at the time of sampling as well as in percentages of the original fresh weight. The latter method, involving correction for changes in effective size of sample taken, appears to give a more accurate method of studying the changes taking place during ripening.

3. A study of the sugar changes during ripening showed that levulose increased during the entire ripening period. Sucrose increased for the first six to eight days and then decreased throughout the rest of the period studied. Dextrose remained constant during the formation of sucrose, but increased later as sucrose disappeared. The increases in dextrose and levulose accompanying the decrease in sucrose were approximately equal, and their sum was about the same amount as that of the sucrose hydrolyzed.

4. The principal difference in the sugar content of fruit of the three pickings lay in the differences in the amounts of sucrose present. Throughout the ripening period the early picked fruit showed the least sucrose, the fruit of the commercial picking an intermediate amount, and the late picked fruit the highest values of this sugar.

5. Total sugars, calculated as the sum of dextrose, levulose, and sucrose, increased during the ripening process; this increase was

considerably greater than could be accounted for by the observed decreases in the alcohol insoluble residue.

6. Sorbitol was identified as a constituent of Bosc pears, and a quantitative method was devised for its measurement. Sorbitol was observed to decrease during the ripening process in fruit of all three pickings, and these decreases were large enough to account for the observed increases in total sugars.

The writer wishes to acknowledge his appreciation for the encouragement and helpful suggestions offered by Professors C. A. SHULL and S. V. EATON during the course of this investigation.

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## DIFFERENTIATION OF THE SPIRAL VESSELS IN *RICINUS COMMUNIS*

FLORA MURRAY SCOTT

(WITH TWENTY-ONE FIGURES)

Although *Ricinus communis*, the Castor bean, is well known in botanical laboratories, the coenocytic development of the spiral vessels seems to have escaped attention. The Castor bean is perennial in California, forming a bush 6–8 feet high, which grows freely throughout the year.

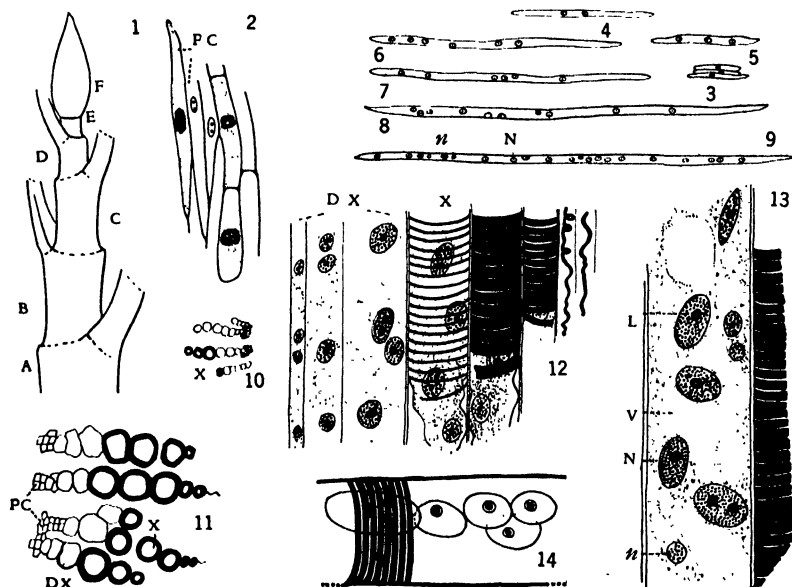
The development of the spiral vessels may be followed in the primary tissues of the young vegetative or flowering shoots. Preliminary observations of fresh material were followed by a detailed examination of serial (imbedded) sections and by a study of macerated tissues. Unless sections are cut at least 12–15  $\mu$  thick, the contents of the developing spiral vessels as a rule escape in sectioning. In tangential sections 30–50  $\mu$  thick occasional coenocytes may be traced throughout the greater part of their length. The method of maceration proves most useful in this connection, for by it complete differentiating elements may be isolated for observation. By this method sections of the stem apex 30–60  $\mu$  thick, after treatment with 5 per cent chromic acid for two to four days, are stained in haematoxylin and mounted in glycerin. Tapping the coverglass with a needle is usually sufficient to separate the vascular elements. The tissues during maceration become extremely fragile, particularly the spiral vessels themselves, and a certain amount of confusion on the slide is therefore unavoidable.

In a typical dictyostele the region of elongation, composed of primary tissues, generally extends through nine internodes. The tip of the shoot, including the first five to six internodes, is inclosed by the characteristic deciduous bud sheath of the genus (fig. 1).

Growth of the shoot involves the usual processes of cell division and cell expansion (6). Microscopic examination reveals apical meristem, procambial cylinder, procambial strands, and ground tissue. The procambial strands differentiate into the vascular bundles of the



axis, leaves and bud sheaths. The first xylem elements to appear are small uninucleate spiral tracheids, two or three in number, at the base of the fourth leaf primordium. The largest spiral elements, in



FIGS. 1-14.—Fig. 1, diagram of young shoot in region of elongation. Lengths of internodes (A–F) and bud sheath: 6.5, 4.0, 2.4, 1.2, 0.6, and 3 cm. Fig. 2, longitudinal section of procambial tissue at base of leaf primordium; mitotic figures, anaphases, present in procambial element ( $45 \times 4 \mu$ ) and in two adjacent parenchyma cells. Figs. 3–8, typical coenocytes from various levels in first seven internodes of growing shoot (fig. 1) from macerated material: 3, 1-nucleate procambial element ( $198\text{--}216 \times 6 \mu$ ); 4, 2-nucleate coenocyte ( $495 \times 7 \mu$ ); 5, 3-nucleate ( $405 \times 8 \mu$ ); 6, 6-nucleate ( $900 \times 12 \mu$ ); 7, 7-nucleate ( $1000 \times 13 \mu$ ); 8, 10-nucleate ( $1350 \times 16 \mu$ ). Fig. 9, 19-nucleate coenocyte ( $1475 \times 27 \mu$ ) from living material, with two smaller possible degenerating nuclei. Fig. 10, transverse section of vessels of vascular bundle from internode E. Fig. 11, vessels of vascular bundle of internode C; diameter of lignified vessel,  $70 \mu$ . Fig. 12, longitudinal section, semidiagrammatic, showing adjacent developing coenocytes. Fig. 13, part of a living coenocyte (diameter  $52 \mu$ ) showing vacuolated protoplasm. Fig. 14, detail of a group of nuclei within a vessel; length of large nucleus,  $34 \mu$ ; of four small nuclei,  $15\text{--}18 \mu$ . PC, procambial tissue; N, nucleus; n, possible degenerate nucleus; L, nucleolus; S, spiral thickening; V, vacuole; DX, differentiating xylem; X, xylem.

the older internodes beyond the bud sheath, are multinucleate vessels whose maximum length is difficult to determine. Some may ex-

tend throughout the entire internode, their endings obscured in the tissues of the node. In any case the spiral thickening is continuous throughout the length of the vessel, and is uninterrupted by any trace of septa. The stages in the development of the coenocytic spiral vessels may be observed in the successive internodes of the region of elongation.

The first indication of differentiation of the xylem occurs in the procambial strands as they become defined below the meristem of the stem apex. Certain cells increase markedly in length and in diameter. Spiral thickening and lignification follow shortly, and result in the formation of uninucleate spiral tracheids at the base of the fourth leaf primordium already mentioned. Throughout the region of elongation a certain amount of cell division is going on constantly in vacuolating dividing cells—prophase, metaphase, and anaphase being most frequently noted (fig. 2). Cell wall formation does not always follow nuclear division, and binucleate coenocytes appear in the procambial strands (fig. 15). This coenocyte lies adjacent to spiral tracheids, horizontal in position, part of the vascular complex supplying the leaf base and bud sheath.

Where the differentiated vascular bundles begin to be established in the region of the fourth and fifth internodes, 3-nucleate and 4-nucleate coenocytes commonly appear in longitudinal sections, particularly where the leaf traces curve inward to join the main axis. Since as a rule the endings of these coenocytes pass out of the plane of the section, their length and complete nuclear number are seldom obtainable in sections, but they may be observed in macerated material (figs. 3-9). This series of diagrams illustrates typical stages in coenocytic development at different levels in the first seven internodes. A gradation in size and in nuclear number is apparent in passing from the stem apex downward. The measurements of the initial procambial elements and of the coenocytes illustrated are given in table 1.

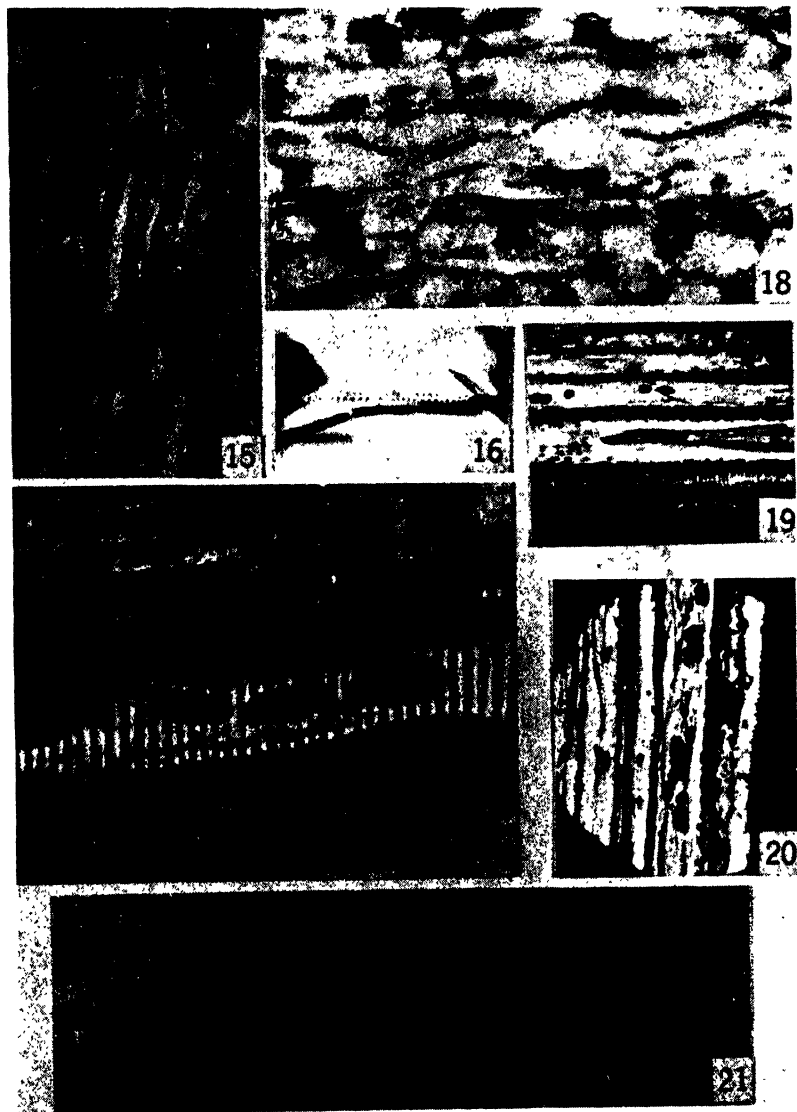
During primary growth the vascular bundles in successive internodes increase markedly in size of vessels and amount of lignification, although not in number of component elements seen in transverse section (figs. 10, 11). The vessels lie in radial series separated by xylem parenchyma. The final stages of differentiation of the larger

spiral vessels are generally best seen in longitudinal sections of the seventh or eighth internodes of the region of elongation (fig. 12). The youngest and least differentiated coenocytes, at the outer margin of the xylem strand, next to the future cambial zone, are narrow, thin walled vacuolating elements. A gradual increase in volume of lumen and of nuclei is seen in the older differentiating stages. The maximum expansion of the coenocyte is reached when its diameter slightly exceeds that of the adjacent lignified vessel. The protoplasm of the living coenocytes at this stage is highly vacuolated, the vacuoles varying much in size (fig. 13). Chondriosomes and granules are abundant, and the presence of small starch grains and a small

TABLE 1

	LENGTH AND WIDTH ( $\mu$ )		NUMBER OF NUCLEI
Procambial cells. . . . .	108-216	6	1
	495	7	2
Coenocytes. . . . .	405	9	3
	900	12	6
	1000	13	7
	1350	16	10

amount of sugar is indicated by microchemical tests. Judging by the visibility of the nuclei, the protoplast may be in a moribund condition at the time of examination. The first trace of spiral thickening appears as a thin cellulose strand generally throughout the whole length of the coenocyte (fig. 16). It is less than  $1 \mu$  wide when first observed, and eventually widens into a close set, heavy band of cellulose,  $2-5 \mu$  deep. Examination in polarized light indicates that the cellulose micels are oriented approximately lengthwise in the spiral band. In transverse section the first traces of lignification appear at the cell corners and along the middle lamella, and may be demonstrated clearly by Maule's reagent, by phloroglucin and hydrochloric acid, or by Lauth's violet, an indicator stain. The spiral, when once lignified, continues to increase in thickness, but cellulose deposition and lignification now keep pace with each other. During this process the living protoplasm remains to all appearance unchanged. I was unable to observe any regular orientation of vacuoles such as has been described by BARKLEY (1), or any regularity



FIGS. 15-21.—Fig. 15, longitudinal tangential section through leaf base; binucleate coenocyte adjacent to differentiated spiral elements. Fig. 16, developing coenocyte in which cellulose spiral is formed (contracted during maceration). Fig. 17, longitudinal section; three nuclei visible within a differentiated spiral vessel. Fig. 18, longitudinal section; anaphase stage of mitosis in a coenocyte. Fig. 19, longitudinal section; contrasting sizes of nuclei obvious. Fig. 20, longitudinal section; close grouping of nuclei apparent. Fig. 21, longitudinal section; part of a 22-nucleate coenocyte. Paired arrangement of four nuclei seen to right.

in the arrangement of mitochondria or particles visible under ordinary conditions of illumination. While lignification is taking place, there is no visible disintegration of the living protoplast. The protoplasm and nuclei of the fully developed vessels remain visible in the lower internodes (fig. 17). The actual time of disintegration of the protoplasmic contents has not been determined.

TABLE 2  
NUMBER AND SIZE OF NUCLEI OBSERVED IN INCOMPLETE  
COENOCYTES IN LONGITUDINAL SECTIONS

	NUMBER OF NUCLEI	SIZE OF PART OF COENOCYTE OBSERVED ( $\mu$ )		SIZE OF ELLIPTICAL NUCLEI: MAJOR AND MINOR AXES
		LENGTH	WIDTH	
Slide no. 6	2	90	8	14×3 27×6 11×3
	3	113	9	14×5 20×5
	3	90	10	14×4 15×4 23×5
	4	136	10	6×5 9×4 15×5
	4	150	12	7×4 7×4 14×5
				14×5
				7×4
				8×4
				11×5
				11×5
Slide no. 15.....5	5	150	14	

It has already been stated that the spiral thickening as a rule appears simultaneously throughout the entire length of the coenocyte. In one preparation, however, one very thin spiral was observed to run only about three quarters the length of the element. The question, therefore, as to whether the spiral actually appears simultaneously, as in the majority of cases, or progressively from one end in its very early stage, as one preparation appeared to indicate, remains an open one.

A striking feature about the nuclei of the spiral vessels is their increase in volume (figs. 14, 19). A typical procambial nucleus in one of the younger internodes (fixed material) measures  $4\ \mu$  in diameter and  $33\ \mu^3$  in volume. Some of the large nuclei in the seventh internode of the same shoot are elliptical in outline, with major and minor sizes  $40$  and  $10\ \mu$  respectively. The calculated volume ( $\frac{4}{3}\pi a^2b$ )  $8373\ \mu^3$  amounts to a 253-fold increase in cubic content. In fixed and stained slides the artifact nuclei appear reticulate in structure, indicating a resting condition. Nucleoli, one, two, or more in number, are constantly present. They are not homogeneous throughout but generally possess a central vacuole of unknown consistency. In fixed material a clear area surrounds the nucleolus.

The increase in nuclear number appears to be due to mitotic division, for in all the material examined no trace of amitosis was observed. Mitotic figures are frequent in the youngest internodes. In the larger coenocytes direct evidence of mitosis is admittedly scanty, for only three figures, one metaphase and two anaphases, were actually found (fig. 18). The variation in nuclear size here, however, may be significant. Where larger and smaller nuclei occur, as they do in about half of the coenocytes examined, it is seen that some of the larger nuclei measure approximately twice the size of some of the smaller, a ratio which may point to intermittent nuclear division. Table 2 gives typical figures.

In fresh material the difference in nuclear size is again apparent, and here some of the larger nuclei are three or four times the size of the smaller. Thus in the longest coenocyte ( $2565\ \mu$  incomplete) examined, of the twenty-three nuclei, eighteen spherical and five elliptical, the measurements are as tabulated on the following page. The significance of the increase in volume of the nuclei will be considered later, in the general discussion. It is noted further that nuclei of the same size often occur in pairs close together in the coenocyte, a fact which may also point to recent division (fig. 21).

In certain coenocytes a few spherical masses of protoplasm occur which stain more or less deeply with haematoxylin. They are about the same size as the smaller nuclei, but they lack a nucleolus and are not always clearly defined in outline. It is possible that they may be

degenerating nuclei. Occasionally in both fresh and stained material the nuclei are clumped together in the coenocyte. This may be an artifact condition produced in the actual cutting (fig. 20).

		DIAMETER ( $\mu$ )	
Spherical	{ 1 nucleus.....	5	
	{ 8 nuclei.....	7	
	{ 2 ".....	8	
	{ 4 ".....	9	
	{ 2 ".....	10	
	{ 1 nucleus.....	27*	
		LENGTH ( $\mu$ )	WIDTH ( $\mu$ )
Elliptical	{ nucleus.....	12	9
	{ ".....	11	7
	{ ".....	14	5
	{ ".....	19	6
	{ ".....	22	11

\* This nucleus contains a large vacuole-like area near the nucleolus and is considered abnormal.

### Discussion

Certain points of interest arise in connection with this account of the development of the spiral vessel: (1) source of lignin; (2) development of the endodermis and entrance of solutes into the xylem vessels of the root; (3) time of lignification; and (4) hypertrophy of the nuclei.

First, the opinion has generally been held and has been stated recently by WEEVERS (11) that lignification is bound up with disintegration of protoplasm. That disintegration may accompany lignification is possible in certain cases, but the present observations show that the two processes are not necessarily interdependent.

Following from this, in the second place, an interesting point arises in connection with the development of the endodermis, a tissue of maximum importance in the actively absorbing root. PRIESTLEY and NORTH (6) have suggested that the suberin of the Casparian strip is the result of the breakdown of the protoplasts of the developing vascular elements, and this idea has passed into current acceptance. A re-examination of root structure appears to be necessary in order to define the relative time of development of spiral thickening and of

disintegration of the protoplast. This will determine whether the process of lignification is essentially the same or fundamentally different in root and in shoot. In the same paper (6) the question is raised of the entry of solutes from a living cell into an empty xylem vessel. If the protoplast of the spiral vessel is virtually present after lignification, then the osmotic forces of living cells alone are functional in the absorptive region of the root, and the question of entry into the dead vessel automatically disappears.

Third, another question of interest is the time required for the development of the spiral vessel, in particular for the laying down and the lignification of the spiral. It is known that the dividing wall in an algal cell is laid down in from two to three hours (10). The development of the spiral vessels takes place in rapidly growing internodes. In sectioning material it is seen very soon, as has been emphasized by BARKLEY (1), that certain stages in the process of lignification are rarely seen. There is no difficulty whatever in finding either expanding vessels with unthickened cellulose walls, or fully lignified spiral vessels. The intermediate stages of semi-lignification, however, are few and far between. The process of wall thickening and of initial lignification is therefore presumably a relatively rapid process. Since a photosynthetic plant is normally periodic in its functioning, it is probable that the process of lignification is also periodic. Limited data indicate that most active lignification occurs between 1 A.M. and 5 A.M.

Fourth, the hypertrophy of the nuclei in the developing vessel is of direct interest to the cytologist. GUILLIERMOND (3) states that nuclei in higher plants range from 5 to 50  $\mu$  in diameter. The enormous size of the egg nuclei of the gymnosperms and the cycads is well known, of course, 500–600 being usual dimensions. CHAMBERLAIN found in *Dioon* a nucleus measuring  $1475 \times 380 \mu$ . GUILLIERMOND quotes BUSCALIONI in regard to the size of the endosperm nuclei of *Lupinus* which measure  $300 \times 500 \mu$ . But data in regard to the change in nuclear size within the same plant are meager. GOLDSTEIN (2) describes and figures hypertrophied nuclei in the xylem parenchyma and in the storage cells of a normal *Lilium* bulb. In this case the nuclei pass through a lobed condition and eventually fragment. ROSEN (9) describes the nuclei of the young epidermal cells



of *Hyacinthus* as elliptical in outline and approximately 15,000 cubic  $\mu$  in volume. The older nuclei are spherical in form and have decreased to 5800 cubic  $\mu$  or less than one-half the initial volume. It is stated as a general rule that nuclei decrease in size in differentiated tissues. KLEINEBERGER (4) interprets this decrease as a loss of physiological activity. If the latter statement is accepted, then the physiological activity of the differentiating xylem elements is presumably highly intensified.

The question naturally arises, is the hypertrophy due to an increase in karyolymph, in chromatin, or in both substances? The variations in nuclear size suggest possible polyploidy, but this can be determined only when sufficient numbers of mitotic figures are available. Since meristematic division is a periodic function in apical meristems, it is not unreasonable to suppose that a similar periodicity may obtain here, in which case at some happy hour in the twenty-four the various phases of mitosis will readily be available to the cytologist. He will then determine whether the nucleus is virtually polyploid, or whether perhaps the nuclear material resolves itself into units resembling the giant chromosomes of *Drosophila*.

### Summary

1. The development of the spiral vessels in *Ricinus communis* may be observed in the young internodes of the growing shoot.
2. Multinucleate coenocytes differentiate from uninucleate procambial cells. These increase in size in successive internodes and may be observed in macerated material. The coenocytes range in length from 90 to at least 2500  $\mu$ , and the maximum number of nuclei observed is twenty-three.
3. As the developing vessel expands, the cell wall does not thicken measurably. When the maximum expansion is reached, a fine spiral of cellulose is laid down throughout the entire length of the vessel. This increases in thickness and begins to lignify when about 2  $\mu$  wide (in transverse section).
4. The development of the spiral and the initial lignification is presumably a rapid process, since the intermediate stages of semi-lignification are relatively rarely observed. Lignification is presumably a periodic process and may occur most actively in the early

morning. The lignified spiral continues to thicken, but at this stage cellulose deposition and lignification keep pace with each other.

5. The protoplasm of the developing vessel does not visibly disintegrate during lignification, so that this process is not dependent on any visible breakdown of the protoplasm. This fact raises a question as to the source of the suberin and lignin materials in the endodermis of the root, and also entails a reconsideration of the problem of the entrance of solutes from living cells into allegedly empty spiral elements of the root hair region of the root.

6. Whether the hypertrophy of the nucleus is due to an increase of chromatin material or of nuclear sap, or of both, remains undetermined.

7. The question of polyploidy or of giant chromosomes in the hypertrophied nucleus remains for future solution.

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# MORPHOLOGY OF SOME AMERICAN SPECIES OF PSARONIUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 482

NORMAN J. GILLETTE

(WITH TWENTY FIGURES)

## Introduction

Investigations of the genus *Psaronius* Cotta have been numerous, as have been also the investigations of the three form genera *Caulopteris* Lindley and Hutton, *Ptychopteris* Corda, and *Megaphyton* Artis. As early as 1864, STENZEL (25) investigated certain structural aspects of various species of *Psaronius*. ZEILLER (28) studied the fossil flora of the Coal Basin and Permian of Autun and Épinac of France, and described several new species, together with an anatomical description of some. RUDOLPH (23) undertook a comparative investigation of certain species of the genus and some of the living Marattiaceae. In 1906 STENZEL (26) again published a report on the anatomy of certain species, together with a taxonomic review of the more common members of the genus.

Most of the investigations have been made on the European species, and relatively little has been done with the American material. In several instances specimens described as species of *Psaronius* (3, 4, 5, 10, 11, 12) were shown later to be either examples of other genera (8) or the descriptions were altogether inadequate for recognition (17). LESQUEREUX (18) described *Caulopteris giffordi* from Peoria County, Illinois. This specimen was also referred to as *Psaronius giffordi* by POTONIE (22), although no thorough investigation of its structure had been made. FARR (7) described in great detail a fern stem that was collected in Iowa, and appended to his description that of *P. borealis* by MACBRIDE (19) for portions of a stem from the same horizon. Although FARR did not state that his specimen belonged to the same species as MACBRIDE's, the fact that he included in his description that of MACBRIDE indicates that perhaps he considered these two as belonging to the same species.

The roots of *Psaronius* occur commonly in many of the American coal balls (2, 14, 15, 27), but it is only rarely that any stem remains are found in this type of preservation. GRAHAM (9) and NOÉ (20, 21) both mentioned stems of *Psaronius*, but these were not well enough preserved to permit study. Poor preservation is not unusual in coal balls, and the stem size of this tree fern is large. HOSKINS (16), however, described an incomplete specimen of *Psaronius illinoensis* from "a calciferous petrification from the McLeansboro formation of Illinois." This specimen consisted of a narrow zone of roots and a few vascular strands of the stem axis. It was not possible to ascertain the nature of the stem in the living condition, nor could it be associated with any of the larger groups of *Psaronius*.

The several species have been separated into two main groups by HIRMER (13), *Distichi* and *Polystichi*, either on the basis of the number of rows of leaves they possessed or on the number of foliar bundles at the periphery of the stem in a given transverse section. A third group, the *Incertae*, includes those imperfectly known species of which a part or all of the transverse section of the stem is unknown. To the *Psaronii distichi* belongs the genus *Megaphyton*; to the other group *Caulopteris* and *Ptychopteris*. Some species of *Caulopteris* and *Ptychopteris* based on fragmentary specimens would belong to the *Incertae* if they showed any internal structure.

The *Psaronii polystichi* have been subdivided into the *Verticillati* and *Spirales* (13); the species in the former group had their leaves in whorls whereas those in the latter possessed spirally arranged leaves. The main characters used to differentiate the species possessing spirally arranged leaves have been the number of leaf trace bundles appearing at the periphery of any transverse section of the stem, the presence or absence of gum canals in the parenchyma (especially of the woody body), the presence or absence of lacunae in the cortical parenchyma of the roots as well as in the ground parenchyma of the stem axis, and the presence or absence and completeness or incompleteness of the sclerenchymatous sheath tissue in and around the woody cylinder.

The plant material described in this paper represents the silicified remains of Paleozoic tree ferns belonging to *Psaronius*. The specimens were collected about 25 years ago by Mr. VIRGINIUS CHASE of

Peoria, Illinois, in a gravel pit in the vicinity of Peoria. This gravel pit has been abandoned long since. Its geological horizon is not known, but it was probably Pleistocene, and thus these fossils were not found in the strata in which they were originally preserved but were carried to that place by a glacier. Inasmuch as most of the European species of *Psaronius* are Permian, it is possible that these were also, although some species have been described from the Carboniferous. One specimen (no. 32) was collected by Mr. CHASE in Stark County, Illinois, and this may represent a Carboniferous species. The material was obtained from Mr. CHASE by Dr. A. C. NOË while the writer assisted him in field work for the Illinois State Geological Survey in the summer of 1935. The specimens are in the possession of the Peoria Academy of Science, and the number after each species refers to the accession number in that collection.

Since these stems were silicified and etching with hydrofluoric acid did not prove successful, the "peel method" of examining them was not possible. Thin sections were prepared, and the polished surface of the rocks examined. The model of the vascular skeleton of *P. septangulatus* Gillette (no. 2) was made from the study of a series of ten sections through a given stem, each section being about 1 cm. in thickness. The more detailed observations of the tissues were made on the thin sections.

*Psaronius septangulatus* sp. nov. (nos. 2, 5) Figs. 1-12

DIAGNOSIS.—In transverse section seven peripheral bundles alternate with seven foliar bundles. Within this zone several (17-20) strands are arranged more or less spirally. Definite sclerenchymatous sheath around woody body, interrupted opposite divergences of the leaf trace bundles. Ground parenchyma of stem axis dense with scattered gum canals. Leaf scars scattered, 2-2.5 cm. wide, 4-5 cm. high, elliptical, open at base and indefinitely closed at top. Vascular bundle scar large (2.5 cm. long, 1 cm. wide), horseshoe-shaped, in upper two-thirds of leaf scar. Leaves spirally arranged. Phyllotaxy 2/7.

Specimen no. 2 is a portion of a stem 27 cm. long, which in the living condition had a diameter of at least 11.5 cm., including the root zone, although in the state of preservation it is crushed so that its present diameters are 13 and 9 cm. The vascular portion of the



FIGS. 1, 2.—Fig. 1, transverse section of *P. septangulatus* (specimen no. 2) showing vascular cylinder surrounded by zone of adventitious roots. Fig. 2, surface view of *P. septangulatus* (no. 5) showing several leaf scars.

stem itself measures 9 by 5 cm. in diameter. There are few external distinguishing characters. The entire stem is covered with adventitious roots; no leaf scars are discernible. The vascular bundle scars of the leaf, as observations of the sectioned material show, are approximately 2.5 cm. long, 1 cm. wide, about 8.5 cm. from center to center on the same vertical row, and the vertical rows 3-3.5 cm. apart. The roots, somewhat intertwined around one another down the stem, give the surface of the stem a fluted appearance. The trees probably reached a height of over 10 meters (13), and the lower portion of the trunk was covered with a dense growth of adventitious roots.

Specimen no. 5 (fig. 2) is a flattened stem about 9 by 5.5 cm. in diameter and 19 cm. long. The petiolar scars, definitely marked on one side of the specimen, are separated from one another by about 2 cm. or less in the same vertical row and by about 2 cm. from side to side. The scar corresponding to the leaf bundle is elliptical, but the upper portion is open and its edges curve inwardly on themselves. The lower portion of the vascular scar is elevated above the surface of the rest of the leaf scar. The surface of the stem contains scattered pits which probably correspond to the divergences of the adventitious roots. These pits are especially prominent on the ridges between the leaf scars. Because of its external nature the specimen is associated with the genus *Caulopteris*.

The peripheral bundles are rather strongly arched, with their edges curved inwardly. The tissue surrounding the xylem portion of the vascular strands is differentiated from that of the ground parenchyma. This was probably the phloem, although it is very poorly preserved, and it appears to encircle the xylem. The protoxylem is probably represented by groups of small cells on the inside of the vascular bands (fig. 12). Thus the stem would have been endarch. The parenchyma in which the vascular strands are imbedded is somewhat loosely arranged, although it is rather definitely not lacunar. Occasionally there are small groups of sclerenchymatous tissue near the periphery of the stem. There is no definite sheath, but if there had been one external to the peripheral bundles it probably would have been weathered away. Some of the parenchymatous cells are much larger than others, the larger ones con-

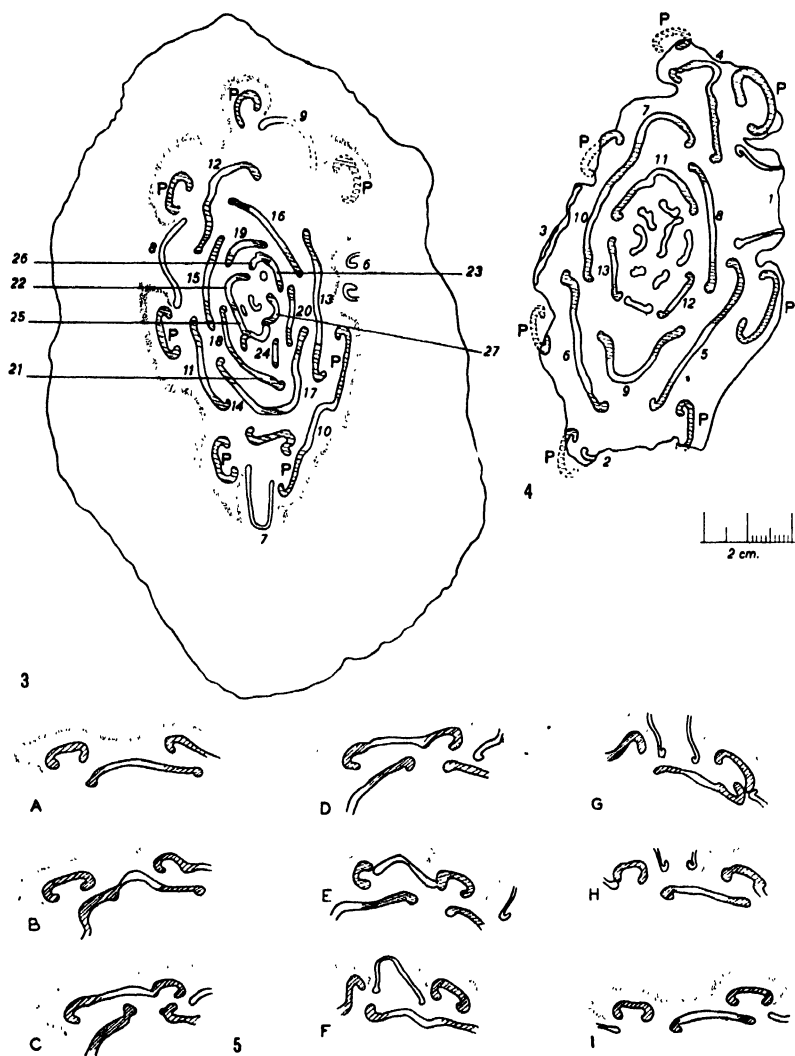
taining some deposited substance. These represent either tannin cells or the so-called gum canals. In general the cellular nature of the vascular tissue is better preserved in this specimen than in no. 2.

This species most closely resembles *P. spissus* Stenzel, but it may be distinguished from it by its greater number of peripheral bundles, five in *P. spissus* and seven in *P. septangulatus*. The woody body of the former species is somewhat smaller in diameter, and the peripheral bundles are separated from the ones on the inside by sclerenchymatous plates. This species differs from *P. demolei* Renault, *P. haidingeri* Stenzel, and *P. espargeollensis* Renault in the non-lacunar nature of the parenchyma as well as in the number of peripheral bundles.

VASCULAR ANATOMY.—(This description is based largely on observations made on specimen no. 2.) In transverse section (figs. 1, 3) the vascular portion of the stem shows a number of conducting strands imbedded in the ground parenchyma of the axis and which seem to be of at least three different types. Around the periphery of the vascular cylinder there are fourteen strands. These are alternately seven peripheral bundles (Randbündel of STENZEL) and seven foliar bundles (Blattbündel). At some levels, however, some of the peripheral bundles and foliar bundles are non-diverged from one another so that there may appear to be fewer than fourteen strands. Within this outer zone of vascular strands there are about sixteen other bundles (the Ersatzbündel and Innere Leitbündel of STENZEL). In transverse section these strands are elongate, platelike, more or less arched toward the center of the stem, and are arranged somewhat spirally in about five coils. Toward the center of the axis they become shorter in their transverse direction, and they are more closely associated. The small strands in the very center seem to have diverged from adjoining strands. In this way it is assumed that new leaf trace bundles may have arisen at the center of the stem. An examination of a series of transverse and longitudinal sections shows that the vascular system is constructed of a network of vascular strands anastomosing and diverging at various places (figs. 6, 7). The significance of these anastomoses and divergences is discussed in detail later.

The peripheral bundles are arched toward the center of the stem,





FIGS. 3-5.—Fig. 3, transverse section of *P. septangulatus* (specimen no. 2) showing vascular cylinder. Leaf trace bundles unlined and numbered 6-27; peripheral bundles (P) and other cauline bundles lined. Sclerenchymatous tissue stippled. Root zone outside of sheath. This drawing is from same transverse section as fig. 1 and represents level at top of schematic diagrams of figs. 6 and 7. Fig. 4, transverse section of *P. septangulatus* (no. 5) showing vascular strands. Peripheral bundles (P) and other cauline strands lined, foliar strands unlined. Bundles shown by broken lines represent reconstructions. Figs. 3 and 4 both drawn to same scale. Fig. 5, series of diagrams showing divergence of leaf trace from zone of steles just inside peripheral zone to base of petiole. Cauline strands lined, foliar parts unlined, sclerenchyma stippled.

and their lateral edges are strongly incurved. These measure about 8 to 15 mm. along the circumference of the stem. The part these bundles play in the formation of the foliar strands will be considered later.

Alternating with the seven peripheral strands are the foliar bundles. The edges of these latter may still be non-diverged from the

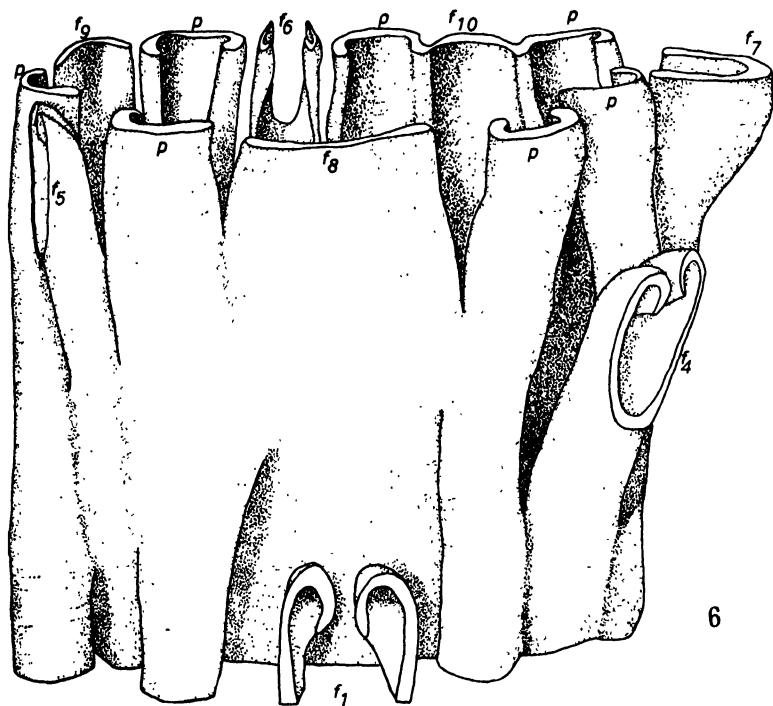


FIG. 6.—Schematic diagram of peripheral portion of vascular skeleton of *P. septangulatus* showing anastomoses of leaf traces with peripheral steles and subsequent divergence into bases of leaves. Peripheral bundles, *P*; foliar strands, *f*<sub>1</sub>, *f*<sub>4</sub>-*f*<sub>10</sub>.

peripheral bundles in certain of the transverse sections. In the peripheral region of the woody body any foliar bundle may vary in form, from a plate parallel to the circumference of the stem, to a U-shaped strand with its free ends toward the stem center, to finally two apparently separate strands with the innermost edges curved toward each other, forming J-shaped strands (figs. 1, 3). The appearance of the foliar strands in transverse section depends on their

degree of divergence from the stem axis. The petiole bundle is horseshoe-shaped with its free edges curved inward (*cf. Stipitopteris*). Each foliar bundle forms an arc with a diameter of 8 to 15 mm., so that this measure corresponds to the width of the vascular

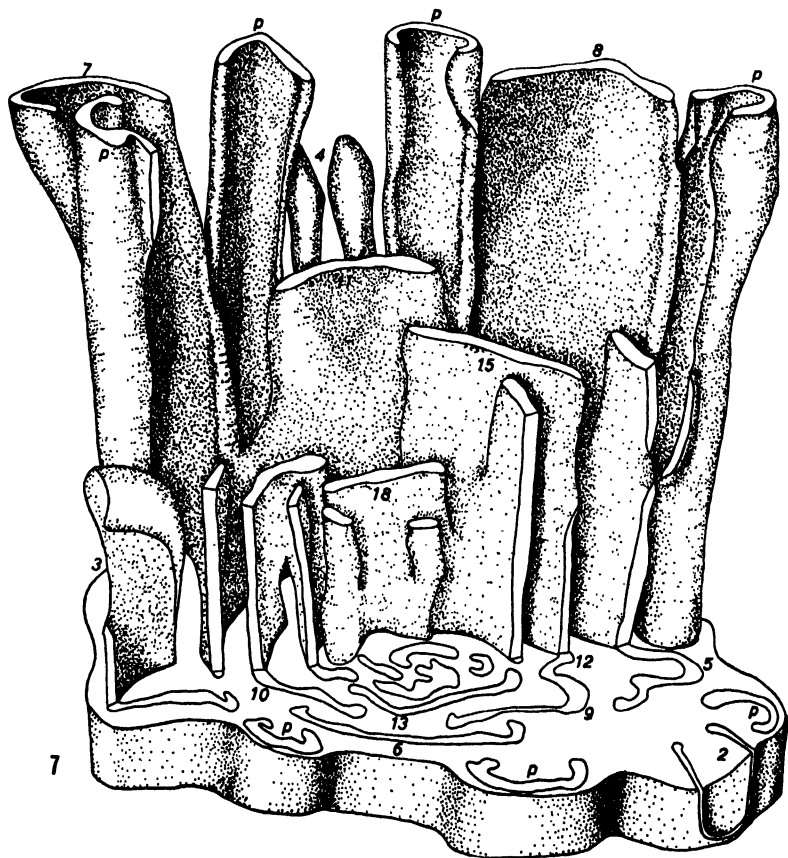


FIG. 7.—Same as fig. 6, but seen from inside. Vascular tissue dissected away from half of stem to show anastomoses and divergences of foliar strands with cauline strands as these former follow their course outward and upward. Peripheral bundles, *P*; foliar traces numbered with Arabic numerals.

bundle scar of the leaf scar, were it not obscured by the growth of adventitious roots on the outside of the stem. It is possible in this specimen to trace the foliar strands almost to the center of the stem axis. The final divergence of each strand is from the two adjoining

peripheral bundles, but it is clear that before this trace anastomosed with these peripheral strands it diverged from vascular plates lying adjacent to and inward from the peripheral bundles. In its divergence from the Innere Leitbündel (fig. 5) a gap is produced, so that in a section made at a higher level this strand with its gap has the appearance of two separate strands. The gap formed by this trace is made complete by the anastomosis with the leaf trace of the leaf immediately above this one leaf, as this subsequent leaf trace diverges outward from the next inner conducting strands. This formation of leaf gaps by the divergence of leaf traces from the conducting strands and the subsequent completion of these gaps by the anastomoses of the traces of the next higher leaves and the side flanks of the vascular strands continues as the leaf traces diverge outward and upward from near the center of the stem axis. Since there are about five coils in the spiral formed by the vascular bands of the woody body, there are five of these anastomoses and divergences before any given leaf trace bundle reaches the stem periphery. The side flanks of these Innere Leitbündel that do not diverge outward, together with the Randbündel, form the cauline system which continues up the stem.

In growth and maturation of the plant tissues, and especially of the vascular tissue, it seems probable that the center of the stem axis remained undifferentiated long after the more peripheral parts at that level were mature. Thus the undifferentiated and partially meristematic region of the stem axis was in the form of an inverted cone. In this way it is possible to explain the fact that the leaf bundles can be traced so far down the stem, as one follows them to the center of the woody cylinder. If the situation were as pictured, the new leaf trace bundles would arise probably as branches from the innermost complex of vascular strands (or provascular strands at the time of their origin). The angle which this leaf trace makes with the stem axis as it diverges outward and upward is approximately  $7^{\circ}$ . This means that the leaf trace originating at the center of the stem at any given level will diverge into the petiole approximately 27 cm. higher up the stem, since the diameter of the woody cylinder may be taken as 7 cm.

The origin of the foliar bundles in this species differs somewhat

from that described by ZEILLER (28) for *Psaronius infarctus* Unger var. *hippocrepicus*. According to him the foliar bands always leave the anastomosis of the vascular bands of the woody body nearest the periphery. This of course is as in the present species, but in the specimen examined by ZEILLER it was impossible for him to trace these strands further toward the center of the stem than just this peripheral region. In this case the leaf trace is described as consisting originally of two distinct halves which have their origins in the anastomoses of the peripheral bands, or a branch from these, with one of the bands at the edge of the central region. These two halves are then united into a single plate, the foliar bundle. STENZEL (26), on the other hand, described another variety of the same species, *P. infarctus* var. *quinquangulus*, in which the leaf trace bundles could be seen as portions of the conducting strands of the region just inside the peripheral circle of bundles; in fact it might be possible to follow a given trace even further toward the stem center. RUDOLPH (23) described a condition that supported STENZEL's conception that the anastomoses appearing between the inner vascular strands probably represented the deeper parts of leaf traces. He described three types of vascular bundles in the stem: the foliar strands, other strands running vertically through the stem (these form a part of the cauline system as defined here), and the so-called peripheral bundles of ZEILLER. RUDOLPH described the course of the leaf trace strands as series of anastomoses and divergences from these cauline strands, in a manner similar to that here described.

HIRMER (13) described three types of leaf trace divergences in the *Psaronii polystichi* which possessed whorled leaves, the *Verticillati*. In the one type, for which he used *P. bibractensis* Renault as the example, the leaf trace is derived from the middle of a bundle plate, the side flanks of which form the peripheral bundles of the woody cylinder at the level of divergence of the leaf trace into the petioles. He described these peripheral bundles as ending blindly and disappearing at the edge of the stem. HIRMER thus pictured the vascular tissue as a series of funnels placed one inside the other, each cycle of leaf traces and their associated cauline bundles representing one funnel. In the second type, that of *P. infarctus* Unger, he found supplementary bundles that had no part in the formation

of the leaf trace. The foliar bundles in this type, however, arose in a manner very similar to that just described for *P. bibractensis*. There are thus alternating "secondary funnels" of vascular tissue which contain no part of the foliar system, but which are closely associated with it, and the "primary funnels" which contain the leaf traces. The third type is exemplified by *P. quadrangulus* Stenzel, and it behaves almost the reverse of *P. bibractensis*. In this case the foliar bundle is formed from the edges of the bundle plates, the central portion of each plate forming the peripheral cauline part of the system. Each leaf trace bundle in this case is therefore composed of two halves derived from as many bundle plates of the stem axis. These two halves unite in pairs at their edges and form together a single horseshoe-shaped leaf trace. In this type, as in the other two just described, the whole vascular system is in the form of funnels placed one inside the other.

FARR (7) described a species of *Psaronius* (*P. borealis* Macbride?) from the Upper Carboniferous of Hardin County, Iowa, which possessed eight longitudinal rows of leaves which he stated appeared to be spirally arranged. It appears from his figures, however, that the leaves were probably arranged on the stem in alternating whorls of four leaves each. The description given by FARR agrees only in the larger details with the specimen here described. He stated that it is apparent "that all leaf traces originate from a central strand and after more or less anastomosis proceed individually to their respective leaf bases. . . . In their course they fuse laterally with the leaf traces of the whorl immediately above and that immediately below. In this way two concentric vascular rings are seen to be formed enveloping the central strand." Although FARR does not indicate that the cauline system played any role in the formation of the leaf trace strands, except perhaps a part of the central strand, he mentions that a "horse-shoe-shaped vascular strand extends from the base to the apex of the stem" between the rows of leaves. No mention is made of any sclerenchymatous sheath around the woody cylinder of the stem, nor is the nature of the ground parenchyma indicated.

All these species, *P. quadrangulus*, *P. infarctus*, *P. borealis*, and *P. bibractensis*, are members of the group of *Psaronii* which pos-

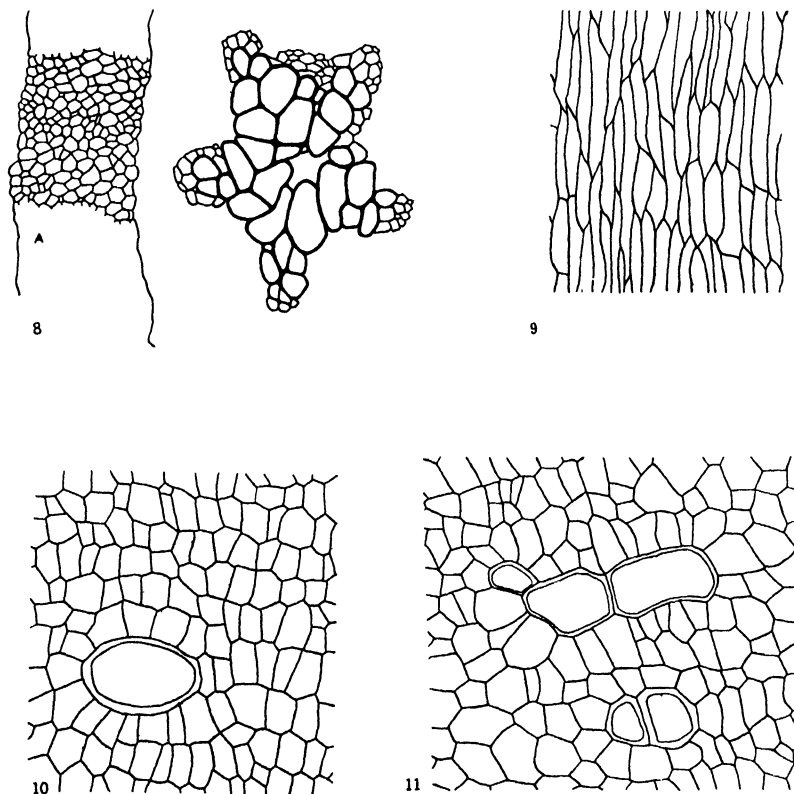
sessed whorled leaves. Little seems to be available concerning the anatomy of those species with spirally arranged leaves. HIRMER is of the opinion that in these species the vascular strands of the woody body are in the form of a "funnel-shaped winding staircase" with the various foliar traces diverging outward as well as upward in the stem. These vascular bands apparently represent the foliar strands as well as the cauline bundles from which these former are derived.

The vascular strands of the stem here described are composed mainly of xylem elements which are rather well preserved in some regions. Where their cellular details are not preserved, they have been infiltrated with a quartz-like material which appears as a contrasting color on the brown ground parenchyma. The xylem is composed of rather large scalariform tracheids which are relatively long with blunt tapering ends. Although no protoxylem has been identified in the longitudinal sections, it is assumed that these stems were either mesarch or endarch, and probably the latter. Around the xylem of the strand there is in some places a tissue that is differentiated from the ground parenchyma. In this region, where any cellular details can be seen they are very small elements with very thin walls. In all probability this represents the phloem region. It is not unusual that this is poorly preserved, because of the nature of the tissue in its living state. In some regions in the stem it is possible to see deposits in the cells of this region, which might represent accumulated food stuffs. This zone of cells seems completely to surround the xylem.

The ground parenchyma of the woody axis is non-lacunar. Its cells are relatively large, thin walled, and isodiametric. Some of the cells contain a deposit of opaque material which may be tannin or some other accumulated plant product. Although there are no lacunae, the cells are not so compact that they form sharp angles in their corners; the reverse is the case, for the cells seem so loosely arranged that each is nearly spherical. These cells vary only slightly in size.

Surrounding the central vascular region is a sheath of sclerenchymatous cells. These are much smaller than either the ground parenchyma of the axis or the xylem tracheids (figs. 8A, 9). Its cells

are compact, somewhat angular, and have thick walls. In longitudinal section they are somewhat elongate and tapering but not nearly so long as are the tracheids. The cells of this sheath are not clearly marked off, either from those within or from those immediately out-



FIGS. 8-11.—Fig. 8, stele of pentarch root of *P. septangulatus* together with some sclerenchymatous tissue of outer cortex of root (A). Fig. 9, longitudinal section of some of cells from sclerenchymatous sheath of *P. septangulatus*. Fig. 10, transverse section of parenchymatous tissue found outside of some of peripheral strands and in leaf scars of *P. septangulatus*. Tissue has appearance of a periderm, and in it are found numerous gum canals. One is shown here. Fig. 11, longitudinal section of same tissue as shown in fig. 8. All figures drawn to same scale.

side it. The sheath is not continuous in the region of the divergences of the leaf trace bundles from the axis.

The roots that surround the woody cylinder are numerous and



pass down the stem in somewhat vertical rows, although there is some intertwining. The steles of these roots are pentarch or hexarch. The xylem in some is rather well preserved, with the large metaxylem in the center and the protoxylem toward the outside (fig. 8). The phloem and the inner cortical parenchyma are not well preserved, but in those regions where some of the inner tissue is preserved it appears to be non-lacunar. The outer region of the root is of thick walled sclerenchymatous cells. These are of the same type as are the cells of the stem sheath; in fact the tissue of the sheath of these roots first appears associated with the steles in the sclerenchymatous sheath of the stem. The vascular elements of the root are derived mainly from the peripheral bundles of the stem, although some may be seen diverging from leaf trace bundles within the peripheral zone. In the root region of some species it is possible to differentiate two separate zones, an inner one in which the roots are imbedded in relatively dense parenchyma, and an outer zone where the roots are more or less free from one another. This specimen shows only one zone of root development.

The parenchyma outside the sclerenchymatous sheath of the roots, in which these are imbedded, consists of somewhat elongate cells in numerous closely intermingled multicellular filaments, having the appearance of a homogeneous tissue; but the filaments seem "combed" in various directions. Concerning the origin of this parenchymatous tissue there have been two main ideas. STENZEL (25, 26) supposed that the inner portion of the zone was a part of the cortex of the stem itself, the roots being intrusive structures. RUDOLPH (23) likewise thought that the regular arrangement of the roots showed that they penetrated a tissue already formed. FARMER and HILL (6) have compared the root zone of the present day *Marattia-ceae* with that of *Psaronius* and found that the root is an intrusive organ in the former, whereas in *Psaronius* the outer cortical region of small-celled sclerenchyma of the root passes almost insensibly into the parenchymatous tissue between them. SOLMS-LAUBACH (24) has shown that the filling-in tissue might have originated from a cellular proliferation at the stem periphery as well as at the root periphery. BERTRAND (1) likewise concluded that this filling-in tissue was "due to a proliferation of a suberose nature, produced at the expense of the superficial tissues."

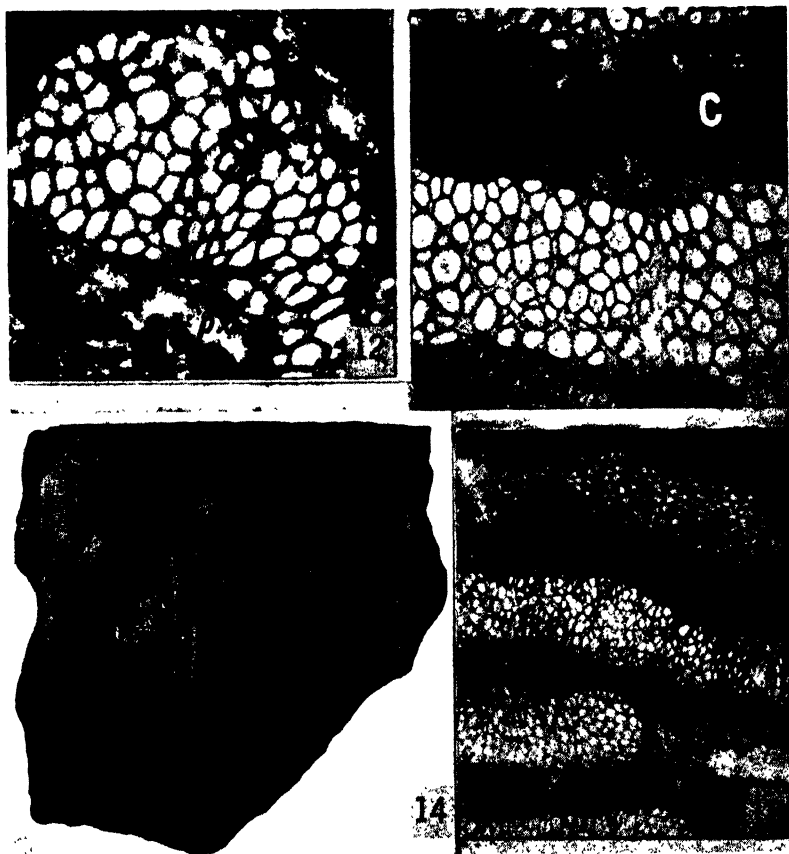
The cells just inside the root zone are differentiated like a periderm (figs. 10, 11). This type of tissue is found especially in the region of a leaf base, but may also be seen outside some of the peripheral bundles. It would appear that in the former case it was formed at the time of leaf fall, or just subsequent to it. It was probably developing at the time the roots were descending on the stem outside it, and by means of this periderm-like growth the irregularities in the stem circumference due to grooves, ribs, etc. were smoothed out by the time the roots were developed at that level. Since the cells in this zone have such a definite orientation, it is possible to detect definite gum canals which could not be identified with certainty in the more loosely arranged ground parenchyma of the woody axis.

*Psaronius peoriensis* sp. nov. (no. 3) Figs. 14-16

DIAGNOSIS.—In transverse section seven peripheral strands alternate with seven foliar strands; within the peripheral zone about forty vascular strands. No definite sclerenchymatous sheath, but regions of sclerenchymatous tissue associated with the outer vascular strands. Parenchyma of woody body somewhat loose but not lacunar; gum canals scattered and few in number. Leaves rather separated; leaf scars subcontinuous, elongate, tapering at both ends, 10 cm. long, 2 cm. wide. Vascular bundle scar centrally located in leaf scar. Leaves spirally arranged. Phyllotaxy 2/7.

Specimen no. 3 is a flattened stem fragment 8 by 4 cm. in diameter and about 10 cm. long. The scars of the petioles on the external surface are arranged in approximately longitudinal rows. The scars corresponding to the vascular bundles are elliptical, 10-14 mm. wide and approximately 4 cm. long. These are probably open at their upper end with their free ends curved inwardly. The vascular scar is located about in the center of the leaf scar. The scars in the same vertical row are almost joined end to end, the rows themselves being 4 cm. apart from axis to axis. The surface of the stem between the scars is not smooth, but is covered with somewhat elongated but only slightly elevated warts. The verrucose nature can also be seen in some of the petiole regions where the outer portions of the scars have been destroyed. The bases of the adventi-

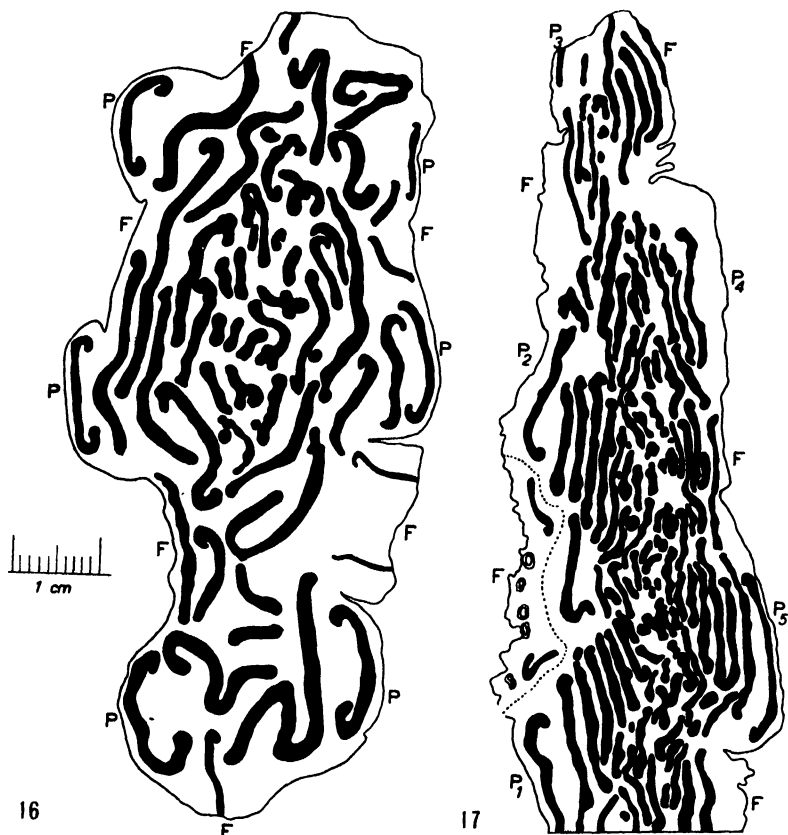
tious roots can be seen in various scattered places, especially in the regions outside the leaf scars.



FIGS. 12-15.—Fig. 12, transverse section of portion of vascular strand of *P. septangulatus* (specimen no. 5) showing protoxylem (*px*); endarch condition. Fig. 13, transverse section of portions of vascular strands of *P. giffordi* showing xylem tracheids. Some tannin cells (?) or gum canals appear as large dark round cells (*C*). Fig. 14, surface view of *P. peoriensis* (specimen no. 3). Fig. 15, transverse section of portion of stem of *P. peoriensis* showing portions of three vascular strands and some of ground parenchyma of axis (*P*).

In transverse section (fig. 16) there are clearly six peripheral bundles, but there were probably seven of these bundles alternating with the seven foliar strands, since the leaves were definitely not

whorled, nor did they have a phyllotaxy of  $1/6$ . The vascular strands within the peripheral zone vary greatly in their transverse direction from circular strands to long bandlike strands. The foliar bundles do not seem to be very closely associated with the periph-



FIGS. 16, 17.—Fig. 16, transverse section of *P. peoriensis*. Peripheral bundles, *P* leaf traces, *F*. Fig. 17, transverse section of *P. giffordii*. Peripheral bundles,  $P_1$ – $P_4$ . Both figures drawn to same scale.

eral strands in their divergence from the woody body; at least in the one transverse section there was no non-divergence of foliar strands from the peripheral strands as in most of the sections of the other species. The protoxylem of the vascular strands appears to be located on the inside of the curved vascular strands, and

in transverse section appears as groups of small cells. On the outside of these vascular bands in some places cells have been identified as phloem cells. These are not well preserved, however, and they are not present on the outer surface of all the vascular bands, although the tissue in that region has a different appearance from that of the ground parenchyma in those where the phloem is not preserved. This region occupied by the phloem is very narrow in comparison to that occupied by the xylem. The larger cells of this region and some of the cells of the ground parenchyma of the woody body appear to be filled with a dense black substance. This may be tannin or some other substance deposited in these cells.

*Psaronius giffordi* Potonié (no. 32) Figs. 13, 17-20

DIAGNOSIS.—In transverse section six peripheral bundles alternate with six foliar bundles. Other strands very numerous, approximately 100, arranged more or less in six groups opposite the six peripheral bundles. Parenchyma. . . . Numerous very large cells which may be gum canals. Leaf scars elliptical, open at the top as a horseshoe, 2-2.5 cm. wide, 4 cm. long, subcontinuous in the vertical rows. Vascular bundle scar in upper two-thirds of the leaf scar, horseshoe-shaped. Leaves in alternating whorls of three leaves each.

This specimen is the crushed portion of a stem showing in all nine leaf scars (which are arranged in six vertical rows) the members of alternating rows apparently forming the various whorls of leaves. Each whorl thus contained three members. The rows of leaf scars are about 4.5 cm. apart from center to center. The scars themselves are less than 1 cm. apart in any vertical row. The vascular bundle scars measure 14 mm. in width and 25 mm. in length. The tissue outside the petiolar scars contains sparsely separated pits corresponding to the divergences of the small adventitious roots. In addition to these larger pits the surface of the stem is finely grained. On one side of the specimen the root zone is still partially intact, especially over the rows of leaf scars. In this region it is possible to see the small adventitious roots imbedded in their matrix. These roots do not run parallel down the stem but are intertwined about one another.

In transverse section the numerous vascular strands are so closely

associated that it is difficult to determine anything about the origin of the leaf traces, etc. (fig. 17). The peripheral bundles around the edge of the stem, however, do alternate with the foliar traces as in the other species described. In general there seems to be more regularity of arrangement of the vascular strands opposite the



FIGS. 18-20.—Figs. 18, 19, views of the two surfaces of *P. giffordi* showing leaf scars. In fig. 19 the root mantle covers the leaf scars. Fig 20, longitudinal section of xylem tracheids of *P. giffordi* showing scalariform thickenings on walls.

peripheral bundles than opposite the foliar bundles. There thus appears in the stem in groups radiating from the center toward several of the peripheral bundles at least four definite groups at  $P_1$ ,  $P_2$ ,  $P_4$ , and  $P_5$ . Because of the crushed nature of the stem this group is not evident opposite  $P_3$ . The portion of the stem including  $P_6$  is not present in this specimen, but it is assumed that there are six peripheral strands because of the apparent location of the center of the stem axis, and because of the whorled arrangement of the leaves.

The general construction of the woody body of this species may

have been similar to that described by HIRMER (13) for *P. infarctus*, in which there were present parallel to the bundle plates giving rise to the leaf traces "secondary bundle funnels" which had nothing to do with the emission of the leaf trace. Because of the nature of the preservation of this specimen it is not possible to determine anything concerning the origin of the leaf traces, as already pointed out. *P. infarctus* was a much larger stem, however, there being five to seven leaves in each whorl, so that there were ten to fourteen peripheral bundles.

The cellular nature of the xylem portion of the vascular strands is especially well preserved (figs. 13, 20). It is composed of relatively large tracheids which have scalariform markings on their longitudinal walls. The woody cylinder was surrounded by a rather indefinite sclerenchymatous sheath, but this had been destroyed in some regions of the stem. In the longitudinal section of the stem cut at right angles to the long diameter of the crushed stem, the vascular strands can be traced the whole length (6 cm.) of the section without anastomosis or branching. The ground parenchyma in which the vascular strands are imbedded is much crushed and poorly preserved, but it appears to have been of a dense nature. In some places, however, there are large spherical cells that seem to contain some sort of deposition substance. These may be what some interpreted as gum canals, or on the other hand they may represent tannin cells.

The external character of this specimen agrees rather closely with that of *Caulopteris giffordi* Lesquereux (18), except for the larger size of the scars of this latter. Inasmuch as no anatomical study has ever been made of *C. giffordi*, the correlation between the internal structures is not possible. POTONIÉ (22) called the internal portion of *C. giffordi* *Psaronius giffordi*. The specimen here described has been compared with the type specimen of LESQUEREUX's species, specimen no. 41006 in the paleobotanical collection in Walker Museum.

### Summary

1. A morphological study of four specimens of the Paleozoic tree fern *Psaronius* was made. These specimens were placed in the following three species: *Psaronius septangulatus* Gillette, *P. peoriensis*

Gillette, and *P. giffordi* Potonié. *P. septangulatus* and *P. peoriensis* possessed seven rows of leaves arranged spirally; *P. giffordi* possessed six rows of leaves arranged in two whorls.

2. The vascular anatomy of one of the specimens of *Psaronius septangulatus* was studied in detail from a series of transverse sections of the stem. The leaf traces diverge outward from the center of the stem axis in a manner somewhat similar to that described for *P. infarctus*, which possessed whorled leaves.

3. Thin sections of all three species were examined and described for the different tissue types.

The writer is indebted to Mr. VIRGINIUS CHASE of the Peoria Academy of Science for the material used in this study. Thanks are due also to Dr. A. C. NOÉ for helpful suggestions and criticism during the progress of the work.

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# MEIOTIC STUDIES IN TRIPLOID TULIPA WITH SPECIAL REFERENCE TO BRIDGING AND FRAGMENTATION

MARK W. WOODS

(WITH THIRTY-ONE FIGURES)

## Introduction

NEWTON (3) and NEWTON and DARLINGTON (4) have called attention to the autopolyploid nature of a number of tulips, and the meiotic behavior in three triploid garden tulips has been described in detail by these investigators (4). The present paper reports studies of microsporogenesis in the triploid cottage tulip, Inglescombe Yellow. Certain types of behavior, not hitherto described for *Tulipa*, have been determined in this variety. Special attention has been given to the formation and behavior of chromosome fragments, and to the distribution of chromatin bodies in interphases I and II. No attempt will be made to review here the extensive literature on chromosome fragmentation, and the occurrence of chromosome fragments in plants. MACMAHON (2) has recently published an excellent review of this literature.

## Material and methods

Anthers were obtained in the fall from bulbs that had been grown one or more seasons in Maryland. Material to be sectioned in paraffin was fixed in Flemming's medium solution or Navashin's fluid. Heidenhain's iron-alum haematoxylin was used to stain the material. Most of the studies were made from temporary smears prepared by the aceto-carmin technique, but permanent smears were also made according to the method of TAYLOR (6). Observations were made with a 90 $\times$  apochromatic oil immersion objective of n.a. 1.30 and a 15 $\times$  compensating ocular. All drawings were done with the aid of a camera lucida.

## Results

### CHROMOSOME BEHAVIOR IN MICROSPOROGENESIS

DIAKINESIS AND METAPHASE I.—Trivalents, bivalents, and univalents occurred in prophase I and metaphase I. Table 1 gives the results of a detailed examination of 53 pollen mother cells in which every chromosome association was determined. The average number of trivalents per pollen mother cell was 9.58, or approximately 80 per cent of a complete trivalent association. Some of the observed

TABLE 1

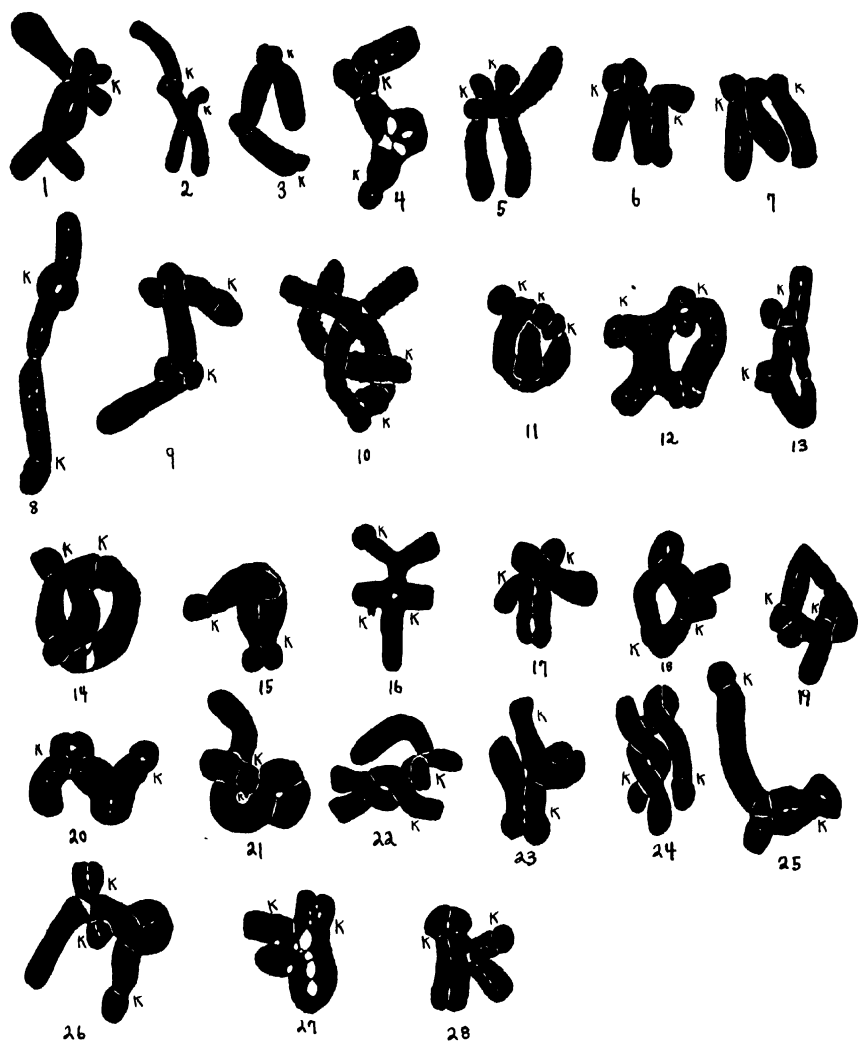
CHROMOSOME ASSOCIATIONS AT LATE DIAKINESIS AND METAPHASE I IN INGLEScombe YELLOW (3N EQUAL 36).  
DATA FOR 53 POLLEN MOTHER CELLS

TOTAL NUMBER OF CHROMOSOME ASSOCIATIONS PER POLLEN MOTHER CELL				
TRIVALENT	BIVALENT	UNIVALENT	OBSERVED FREQUENCY	PERCENTAGE OF TOTAL NUMBER OBSERVED
5.....	7	7	2	3.77
6.....	6	6	0	0.00
7.....	5	5	4	7.54
8.....	4	4	6	11.32
9.....	3	3	7	13.30
10.....	2	2	19	35.83
11.....	1	1	11	20.75
12.....	0	0	4	7.54

trivalent configurations are shown in figures 1-28. In most of the trivalents only two of the homologues were associated at a single point (figs. 3, 6, 13). Sometimes three homologues were closely associated in the region of their kinetochores (spindle attachment regions), although the actual unions may not have occurred between more than two of the chromosomes at a single locus (figs. 1, 5).

Associations like those shown in figures 27 and 28 were rarely observed. In such cases distinct repulsion had occurred between the completely associated homologues and the third chromosomes at both ends, but all three were associated in the middle.

ANAPHASE I.—By late metaphase or early anaphase each homologue was split into two daughter chromosomes. Various irregulari-



FIGS. 1-28.—Trivalent configurations from late diakinesis and metaphase I of microsporogenesis in Inglescombe Yellow. Figs. 5 and 10 from diakinesis; remainder from metaphase.

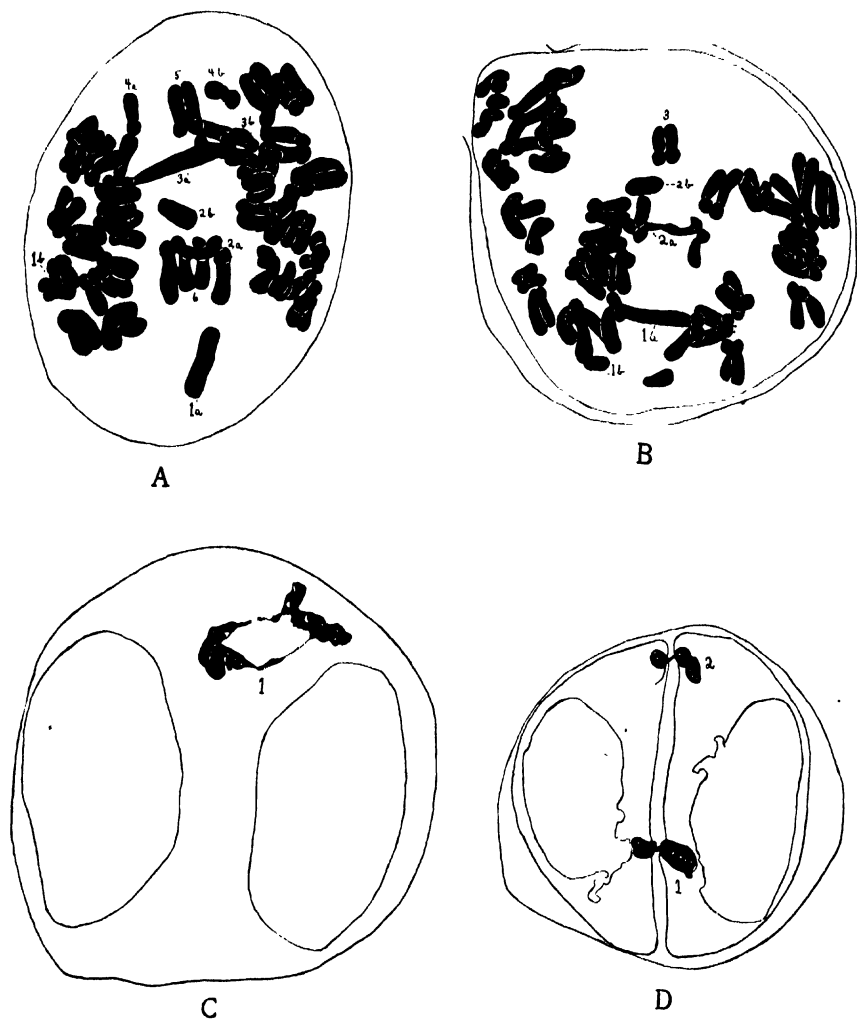


FIG. 29.—Microsporogenesis in Inglescombe Yellow. *A*: anaphase I with three cases of fragmentation and associated irregularities. 1a is kinetochoreless fragment deleted from two daughter chromosomes at 1b. Whole configuration (1b) passed to one pole instead of forming a bridge. A typical bridge with its accompanying fragment is shown at 2a and 2b. Long bridge at 3a is accompanied by very short fragment attached to distal end of daughter chromosome 3b. 4a and 4b are daughter chromosomes derived from single univalent. 5 and 6 are lagging, but divided univalents. *B*: anaphase I with two cases of bridging (1a and 2a) and their accompanying fragments 1b and 2b. Note that fragment 1b is attached to distal end of one of daughter chromosomes. *C*: large mass of chromatin excluded from daughter nuclei in first division. *D*: excluded chromatin fragments apparently being cut in two by developing cell plate.

ties occurred during anaphase. The bivalents most often separated in a regular fashion with one pair of daughter chromosomes going to each pole. The trivalents and univalents, however, frequently behaved irregularly (fig. 29*A*). Table 2 presents an analysis of ten ana-

TABLE 2  
CHROMOSOME DISTRIBUTIONS IN TEN POLLEN MOTHER CELLS OF  
INGLEScombe YELLOW AT ANAPHASE I

NO. OF P.M.C.	DAUGHTER CHROMOSOME PAIRS (POLE A)	SINGLE CHROMO- SOMES (POLE A)	DAUGH- TER CHROMO- SOME PAIRS (POLE B)	SINGLE CHROMOSOMES (POLE B)	LAGGING UN- DIVIDED UNI- VALENTS	LAGGING DIVIDED UNI- VALENTS	OTHER LAGGING CHROMOSOMES
1 . . . . .	17	1	15	1	0	0	1 bivalent
2 . . . . .	16	0	16	1	1	2	.....
3 . . . . .	16	0	16	0	0	4	.....
4 . . . . .	17	2	17	0	0	0	.....
5 . . . . .	14 irreg.?	1	17	2 plus a fragment	1	1	.....
6 . . . . .	14	0	16	1	1	3	1 bivalent
7 . . . . .	18	0	17	0	1	0	.....
8 . . . . .	15	0	18	0	1	0	1 bivalent
9 . . . . .	14 plus a fragment	0	19	1 fragment	0	2	.....
10 . . . . .	17	2	14	0	0	3	.....
Average	15.8	0.60	16.5	0.50	0.50	1.5	0.30 bivalent

phase I chromosome distributions. From this and other data, it has been determined that the following events may occur at anaphase I:

1. One or more of the univalents may fail to divide, and then lag or go to one of the poles.

2. The univalents may divide, after which they may lag, or one daughter chromosome may go to each pole; or both daughter chromosomes may go to the same pole.

3. Bivalents may lag.

4. Various irregular configurations involving bivalents or trivalents may occur. These sometimes form bridges or exhibit other irregular behavior. Fragment chromosomes result in certain cases.

Chromosome bridging and fragmentation have been studied in particular. Typical cases of bridging are illustrated in figure 29*A*, *B*. Figure 30*A* is a photograph of the cell shown in figure 29*A*.

Bridging and chromosome fragmentation are closely associated. A schematic interpretation is given in figure 31. The diagrams in this figure are lettered and numbered to correspond with the actual drawings, which appear in solid black. Metaphase I trivalent configurations like the one shown in figure 31*A* (an enlargement of figure 28) have been observed. The opening out of such a configuration in anaphase could conceivably result in the situation shown in figure 31*B, C, D*. Detailed analysis of a number of cases of simple bridging and fragmentation have shown (*a*) that the length of its

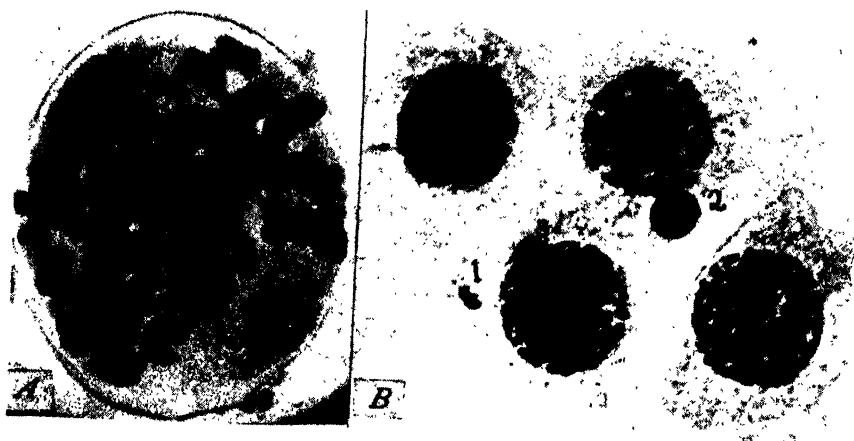


FIG. 30.—Microsporogenesis in Inglescombe Yellow. *A*: anaphase I with three cases of fragmentation and associated chromosomal irregularities (same cell as shown in fig. 29*A*). *B*: tetrad of microspores showing extra nuclear chromatin bodies at points numbered 1, 2, 3.

bridge (fig. 31*C*) is inversely proportional to the length of the fragment lacking a kinetochore (fig. 31*D*); (*b*) that the bridge always has two kinetochores being formed from portions of two chromosomes distally united; and (*c*) that the number of kinetochoreless fragments is equal to the number of bridges or similar configurations. In some cases the fragment is attached to the distal end of one of the daughter chromosomes that is not involved in the bridge (1*b* of fig. 29*B*). Unless attached to another chromosome in this manner, the fragment fails to move in anaphase. The fragments lacking kinetochores have not been observed in a divided condition, although there was very slight evidence of a split in chromosome 1*a* of figure 29*A*.

A situation like that illustrated in figure 29*A* (chromosome groups 1*a* and 1*b*) should result in bridging in the second anaphase. In this instance the breaks in the two homologues occurred very close to the kinetochores; but instead of forming a bridge in anaphase I the whole kinetochore-bearing configuration (1*b*) passed to one pole, leaving the fragment (1*a*) at the equatorial plate.

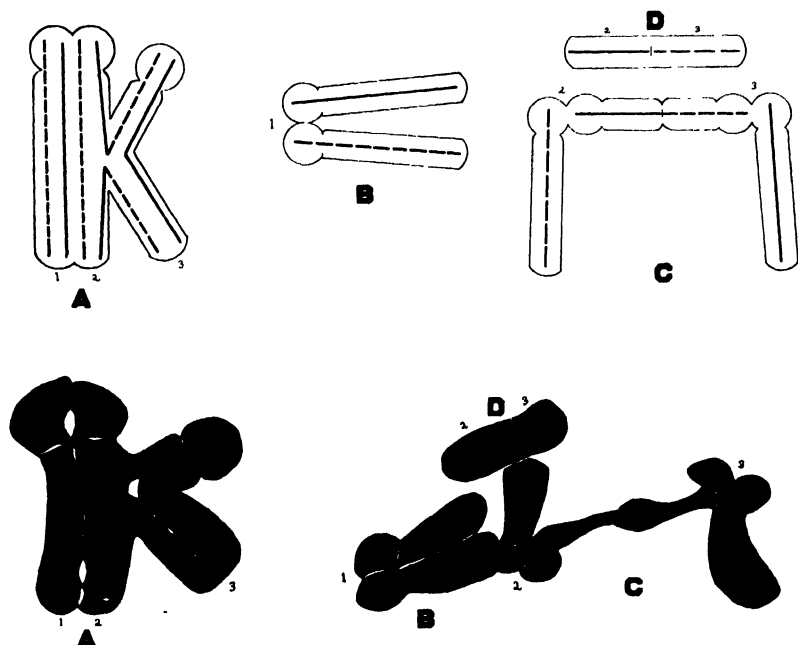


FIG. 31.—Diagrammatic interpretation of simple bridging in Inglescombe Yellow. *A* (solid black): enlargement of fig. 28. Diagrammatic interpretation of this configuration is labeled to correspond with the drawing. *B*, *C*, and *D* (solid black): enlarged from fig. 29*B* (chromosome groups 2*a* and 2*b*). Diagrams labeled to correspond with drawings. In the drawings and diagrams the homologues have been labeled 1, 2, and 3. Each of these becomes double in anaphase as shown in *B*, *C*, and *D*. Solid and broken lines have been used to denote individual chromatids. It is believed that metaphase trivalent configurations, like the one shown at *A*, give rise to anaphase complexes like the ones shown at *B*, *C*, and *D*. A number of cases of bridging have been analyzed in which all chromosomes and fragments were accounted for. Analyses in every case were consistent with the interpretation given in this figure.

**TELOPHASE AND INTERPHASE I.**—Particular attention was paid to the fate of chromosome fragments and other chromatin material not included in the daughter nuclei following the first and second divi-



sions. Such chromatin material is apparently never incorporated into any of the nuclei. By interphase I the excluded chromosomes, or parts of chromosomes, were altered in appearance and displayed strong affinity for stains. Sometimes laggards (in bridges or otherwise) were seemingly cut in half by the developing cell plate (fig. 29*D*). In 75 first interphase dyads the number of extra nuclear chromatin bodies per dyad varied from 0 to 6. The average number per dyad was 2.09, 12 per cent having no chromatin bodies and 4 per cent having six bodies per dyad. These chromatin bodies were the remains of excluded chromosomes or portions of chromosomes. In some cases large masses of extra nuclear chromatin had apparently been derived from whole bivalents or trivalents (fig. 29*C*). Counts of extra nuclear chromatin bodies were limited to those dyads in which situations like those shown in figure 29*C* and *D* did not occur. In other words, only completely separated bodies were counted.

**METAPHASE II.**—Second metaphases were characterized by the irregular form of the chromosomes, which were often similar to the first metaphase types. It was not possible to make accurate chromosome counts at this stage.

**ANAPHASE II.**—Bridging occurred frequently during the second anaphase, the chromatin strands often appearing as though under considerable tension. The extra nuclear chromatin bodies formed during the first meiotic division could be distinguished from the other chromosome elements during the stages of the second division by their form and staining reactions.

**TELOPHASE AND INTERPHASE II.**—After development of the microspore nuclei, the extra nuclear chromatin bodies generally assumed a somewhat spherical shape, staining heavily (fig. 30*B*). Unlike certain triploid tulips, Inglescombe Yellow forms microspore tetrads of quite normal appearance. Typical micronuclei were rarely observed. These were entirely distinct from the extra nuclear chromatin bodies described in this paper. The microspore nuclei of a single tetrad frequently differed in size, but the whole form of the tetrad was rather regular.

The distribution of the extra nuclear chromatin bodies in 2895 microspores from eight anthers of two bulbs of Inglescombe Yellow is given in table 3. The distributions obtained were remarkably uni-

form, even in random samples of only 100 microspores each. For the 2895 microspores recorded in table 3 the average number of extra nuclear chromatin bodies per microspore was approximately 0.8515, or 3.40 bodies per group of four microspores. In the compilation of this table both free microspores and those still organized in tetrads were included. Cytokinesis, however, had been completed in every

TABLE 3

DISTRIBUTION OF EXTRA NUCLEAR CHROMATIN BODIES IN 2895 MICROSPORES FROM EIGHT ANTHERS OF TWO BULBS OF INGLEScombe YELLOW. IN PLANT NO. 1 THE MICROSPORES WERE MOSTLY IN DEFINITE TETRAIDS, BUT IN NO. 2 SOME MICROSPORES WERE STARTING TO SEPARATE. AVERAGE NUMBER OF BODIES PER MICROSPORE WAS 0.8515

No. BODIES PER SPORE	PLANT NO. 1, ANTHERS 1 TO 3			PLANT NO. 2, ANTHERS 1 TO 5					AVERAGE IN PER- CENT- AGE
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 4	No. 5	
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 4	No. 5	
0 . . . . .	40.2	39.5	40.5	36.0	40.2	39.1	39.6	42.5	39.7
1 . . . . .	38.4	39.0	41.5	42.3	36.9	41.3	41.0	40.5	40.1
2 . . . . .	15.4	18.5	14.0	16.0	17.6	14.5	15.6	14.0	15.7
3 . . . . .	4.6	2.5	2.0	4.6	4.5	3.8	3.6	2.5	3.5
4-5 . . . .	1.2	0.5	2.0	1.0	0.6	0.3	0.0	0.0	0.7
Total no. examined	388	400	200	300	487	600	300	220	.....

case. In table 4 the frequency of extra nuclear chromatin bodies in 326 tetrads is given. These tetrads constituted 1304 of the microspores recorded in table 3. The average number of bodies per tetrad was approximately 3.45.

LATER STAGES.—Only limited studies have been made in material collected at a later stage than that immediately following separation of the microspores. In bulbs dug about nine weeks after those recorded in table 3 it was found that the extra nuclear chromatin bodies still persisted. They were best demonstrated in material fixed in Carnoy's fluid and stained in hot aceto-carmin. The bodies were smaller on the average and more homogeneous in structure at these late stages. The data in table 5, while very limited, show that differ-

ent triploids do not have the same frequencies of extra nuclear chromatin bodies in their microspores. It was possible to separate

TABLE 4  
FREQUENCIES OF EXTRA NUCLEAR CHROMATIN BODIES IN 326 TETRADES OF MICROSPORES OF INGLEScombe YELLOW. AVERAGE NUMBER PER TETRAD WAS 3.45

NO. OF EXTRA NUCLEAR CHROMATIN BODIES PER TETRAD	NO. OF TETRADES OBSERVED	PERCENTAGE OF TOTAL TETRADES
0 . . . . .	15	4.6
1 . . . . .	34	10.4
2 . . . . .	52	15.9
3 . . . . .	84	25.7
4 . . . . .	62	19.0
5 . . . . .	36	11.0
6 . . . . .	18	5.5
7 . . . . .	12	3.6
8 . . . . .	6	1.8
9 . . . . .	4	1.2
10 to 12 . . . . .	3	0.9

TABLE 5  
DISTRIBUTION OF EXTRA NUCLEAR CHROMATIN BODIES IN MICROSPORES OF THREE TRIPLOID (3N EQUAL 36) TULIPS. MICROSPORES IN LATER STAGE OF DEVELOPMENT THAN THOSE RECORDED IN TABLES 3 AND 4

NO. BODIES PER SPORE	PERCENTAGE OF TOTAL MICROSPORES		
	INGLEScombe YELLOW	INGLEScombe PINK	CARDINAL MANNING
0 . . . . .	47.0	19.3	84.6
1 . . . . .	44.0	32.9	12.6
2 . . . . .	7.0	24.1	1.3
3 . . . . .	1.0	16.4	0.0
4 . . . . .	1.0	5.4	0.0
5 . . . . .	0.0	0.9	0.0
6 . . . . .	0.0	0.6	0.0
Total no. examined . . . . .	100	310	150

these varieties from one another on this basis alone, even when detailed counts were not made.

### Discussion

The diakinetik and metaphase I chromosome configurations observed in Inglescombe Yellow are of the same general type observed by NEWTON and DARLINGTON (4) in triploid *Tulipa*. These workers found from three to ten trivalents at metaphase in each of the varieties worked with. In Inglescombe Yellow twelve trivalents per pollen mother cell were not rare. This tulip is therefore probably essentially autotriploid.

The trivalent associations occurring in Inglescombe Yellow are generally of the type to be expected on the basis of NEWTON and DARLINGTON's observation that pairing of three homologous chromatids never occurred between all three threads at any one point. Configurations like those shown in figures 27 and 28, however, are hard to explain except on some such basis as that presented in figure 31. It seems very likely that breaks do occasionally occur in chromosome bridges at anaphase I in such a way that kinetochore-bearing fragments are incorporated in the daughter nuclei. In support of this view is the occurrence of a fragment chromosome in a seedling derived from a cross in which Inglescombe Yellow served as the pollen parent.

NEWTON and DARLINGTON (4) observed in polyploid tulips and hyacinths that during the diakinetik contraction small bodies of chromatin began to appear in the nucleus which were still visible at anaphase. These bodies were regarded as chromosome fragments. The origin of such fragments was supposed to take place between diplotene and diakinesis as the result of an unusual strain upon a single chiasma. In Inglescombe Yellow fragments have not been observed until the beginning of anaphase I.

NEWTON and DARLINGTON did not refer to the phenomenon of bridging and fragmentation as being related. Recently, however, DARLINGTON (1) has referred to some unpublished work of URCOTT's on bridging and fragmentation in diploid and triploid *Tulipa*. Some of the figures illustrate conditions very similar to those described in the present paper. SMITH (5) has described a process of chromosome fragmentation and bridging in *Trillium erectum* L. that seems to be very similar to behavior observed in the present study. A crossover between a normal chromosome and one with an inverted segment in the long arm was apparently the cause of the condition in *Trillium*.

In Inglescombe Yellow translocations between homologous chromosomes in trivalent association are apparently responsible for the development of bridges and fragments in anaphase I.

There can be no doubt that the extra nuclear chromatin bodies observed in microspores of Inglescombe Yellow follow a regular distribution (table 3). These bodies are derived from chromosomes and chromosome fragments excluded from daughter nuclei in the first and second meiotic divisions. From a consideration of the number of ways in which these extra nuclear chromatin bodies can originate or be increased in number, it is obvious that a mathematical interpretation of the data in tables 3 and 4 would be difficult. It is significant, however, that the numerical distribution of these bodies in the microspores is highly characteristic. They furnish an index as to the amount of certain types of irregular behavior occurring in the different triploids.

### Summary

1. The cottage tulip, Inglescombe Yellow, behaves cytologically as an autotriploid, twelve trivalents at metaphase I not being rare.

2. In anaphase I chromosome bridges and fragments sometimes occurred. These apparently resulted from translocations in the previous prophase between homologous chromosomes involved in trivalent configurations. The bridges consisted of kinetochore-bearing portions of two homologous daughter chromosomes distally united. The released fragments were without kinetochores and consisted of united portions of the same daughter chromosomes involved in the bridges. Kinetochore-bearing fragments were sometimes formed.

3. Lagging univalents, fragments, portions or all of bridge configurations, etc. were often excluded from the daughter nuclei in interphase I. These formed darkly staining extra nuclear bodies in interphase. In anaphase II further bridging and fragmentation occurred, resulting in the formation of additional extra nuclear bodies. Occasionally during cytokinesis, following both the second and first divisions, some of the extra nuclear masses of chromatin were apparently cut in two by the developing cell plates.

4. The microspores were generally organized in tetrads. The frequency distributions of extra nuclear chromatin bodies in the microspores were characteristic. Approximately 40 per cent of the micro-

spores contained no extra bodies, 40 per cent contained one body per spore, 16 per cent contained two per spore, and the remaining 4 per cent from three to five per spore.

5. It was possible to separate three different triploid tulip varieties on the basis of the frequency distributions of extra nuclear chromatin bodies in their microspores.

The writer acknowledges his indebtedness to Dr. RONALD BAMFORD for valuable suggestions, criticisms, and unfailing interest during the progress of this work.

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## ASCORBIC ACID IN THE AVENA COLEOPTILE

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It has been shown (3, 5, 6) that vitamin C (ascorbic acid) may act as a growth factor in plants. It became of interest to investigate the distribution of this substance in the *Avena* (oats, variety Victory) coleoptile, since this organ has been more extensively studied from the standpoint of growth phenomena than any other plant organ. It was desired to determine the ascorbic acid distribution in the coleoptile and to see whether the content changes when certain external conditions are altered. It was proposed also to investigate the possible relations between the growth effects of auxin, one of the plant growth hormones, and ascorbic acid.

The plant tissues were cut from the seedlings in the darkroom and quickly crushed in 3 per cent metaphosphoric acid, the macerate filtered, the filtrate acidified with 1 cc. 0.01 N HCl, and the ascorbic acid content determined by titration with 2-6 dichlorophenolindophenol (20 mg. per cent) from a microburette. The dye solutions were previously standardized with known amounts of pure ascorbic acid (Roche). After titrating for the reduced ascorbic acid, the solutions were treated with  $H_2S$  to reduce all oxidized ascorbic acid present, and the titrations repeated. The difference between the two titrations gave the amount of oxidized ascorbic acid originally present in the filtrate.

Coleoptiles were removed from 72-hour old etiolated seedlings, the primary leaves removed, and the coleoptiles cut into three sections, apical, middle, and basal portions, each section approximately 10 mm. in length. Their reduced and oxidized ascorbic acid contents were determined as outlined, both on the basis of dry and of wet weight. On the average, one hundred coleoptiles per determination were used. The wet weight determinations are shown in table 1.

It is evident that there is a distinct gradient of concentration of reduced ascorbic acid in the *Avena* coleoptile. It is indicated that there is also a gradient of oxidized, which is opposite that of the

reduced, but this latter point demands more determinations, due to the one exception, namely experiment 3. The dry weight determinations are shown in table 2.

TABLE 1  
DISTRIBUTION OF REDUCED AND OXIDIZED ASCORBIC ACID IN  
AVENA COLEOPTILES (MG. PER GM. WET WEIGHT)

EXPERIMENT	REDUCED ASCORBIC			OXIDIZED ASCORBIC		
	TIPS	MIDDLES	BASES	TIPS	MIDDLES	BASES
1 . . . . .	0.415	0.343	0.294	None	0.076	0.087
2 . . . . .	0.592	0.428	0.303	None	0.028	0.061
3 . . . . .	0.614	0.414	0.313	0.056	0.053	0.047
4 . . . . .	0.500	0.448	0.403	None	None	None
5 . . . . .	0.519	0.434	0.350			
6 . . . . .	0.608	0.555	0.455			
Average . . . . .	0.556	0.437	0.353	0.014	0.039	0.049

TABLE 2  
DISTRIBUTION OF REDUCED AND OXIDIZED ASCORBIC ACID IN  
AVENA COLEOPTILES (MG. PER GM. DRY WEIGHT)

EXPERIMENT	REDUCED ASCORBIC			OXIDIZED ASCORBIC		
	TIPS	MIDDLES	BASES	TIPS	MIDDLES	BASES
1 . . . . .	21.80	17.90	16.95	None	3.00	4.85
2 . . . . .	19.91	18.95	13.97	None	1.25	2.63
3 . . . . .	17.90	20.50	18.65			
4 . . . . .	19.95	19.84	17.52	2.17	2.43	2.32
5 . . . . .	25.50	24.30	22.60	None	None	None
Average . . . . .	21.01	20.29	17.34	0.54	1.67	2.45

The distribution of ascorbic acid in the first internodes of *Zea* seedlings (VAN OVERBEEK, unpublished) is similar to that in *Avena* coleoptiles.

BONNER (2) has presented data showing the distribution of dry weights and protein in the *Avena* coleoptiles. These data are shown in table 3.



From table 3 it is seen that basal sections contain more dry weight per unit length than apical sections. Since BONNER's data show a practical equality of percentage protein distribution per unit dry weight, the ascorbic acid distribution per unit weight of cytoplasm would follow that of dry weight as shown in table 2. From the difference between wet and dry weights, the ascorbic acid distribution per unit weight of cell sap is seen to be identical with that of wet weight determinations.

It has been claimed that ascorbic acid is found in higher concentration in those parts of plants which contain higher amounts of

TABLE 3  
DISTRIBUTION OF DRY WEIGHTS AND PROTEIN IN AVENA  
COLEOPTILE (FROM BONNER)

	TIP 5 MM.	NEXT 6 MM.	NEXT 6 MM.	BASE 13 MM.
	Mg. protein per mm.			
Average of 4 determinations.	$1.10 \times 10^{-2}$	$1.21 \times 10^{-2}$	$1.36 \times 10^{-2}$	$1.46 \times 10^{-2}$
	Mg. dry weight per mm.			
Average of 3 determinations.	$3.9 \times 10^{-2}$	$4.9 \times 10^{-2}$	$5.3 \times 10^{-2}$	$5.5 \times 10^{-2}$
Mean percentage protein...	28	25	25.5	27

chlorophyll (4, 8). The synthesis of ascorbic acid in plants, however, is not dependent upon the presence of chlorophyll and photosynthesis, as will be shown later, but may be formed if photosynthesis occurs. It probably could be formed from hexose added to the medium in the absence of chlorophyll.

The following experiments prove that ascorbic acid may be formed in the absence of chlorophyll. Etiolated *Avena* coleoptiles (1000 plants) from seedlings grown in the darkroom with occasional illumination with orange-red light of wave length no shorter than 575 mμ were extracted several times with hot methanol, the extracts filtered through sintered glass, and the chlorophyll transferred to

petroleum ether. The ether extract was passed through a small Tswett sugar column. A faint green band in the upper part of the chromatogram indicated the presence of chlorophyll. Methanol extracts of apical sections showed a visibly greater amount of chlorophyll than basal sections, showing a distribution gradient of chlorophyll. This fact was borne out by spectrophotometric determinations. Apical and basal sections of 500 coleoptiles grown in (1) the orange-red illumination of the darkroom, and (2) complete darkness, were extracted five times with hot methanol, the extracts filtered through sintered glass, and made up to equal volumes (3 cc.) with methanol. The 3 cc. samples were transferred to a standardized cuvette and the extinction coefficients measured in a spectrophotometer, using a Neon arc as light source, and choosing a line at 6599 Å. The extinction coefficient of a known concentration of pure chlorophyll being known (0.476 for 10 mg. per liter of methanol in the above cuvette), the amounts of chlorophyll could be determined. The tips of coleoptiles grown in the orange-red light contained 0.86 gamma (0.00086 mg.) per gm. of wet weight, while the basal sections contained no detectable chlorophyll (the limits of sensitivity of the instrument being about 0.1 gamma for the 3 cc. samples used). The coleoptiles grown in complete darkness contained no measurable amounts of chlorophyll, although the leaves contained some (4 gamma per gm. as compared with 9 gamma in leaves grown in orange-red light).

Coleoptiles grown in complete darkness were found to contain ascorbic acid, however, the distribution gradient from tip to base existing but the absolute amounts being smaller than in plants grown in the orange-red light or in the greenhouse (see later). Apical sections of coleoptiles from seedlings grown in the complete dark contained 0.165 mg. reduced ascorbic per gm. wet weight, while basal sections contained 0.147. This is about 30 per cent (for tips) of the amounts found in coleoptiles from seedlings grown in orange-red light, and about 20 per cent of the amounts found in coleoptiles from seedlings from the greenhouse (see later). Hence photosynthesis is not essential for the formation of ascorbic acid in plants, but when chlorophyll is present, it is distributed in the same way as ascorbic acid, that is, it is in higher concentration in apical parts

of the coleoptile, as shown by the spectrophotometric determinations.

The seeds of germinating seedlings of *Avena* were separated from the roots and the shoot, and analyzed for ascorbic acid. None was present. The coleoptiles of the same seedlings contained 0.54 mg. per gm. wet weight.

It was just stated that light increases the ascorbic acid content of coleoptiles. This is shown by the data of table 4.

The coleoptiles of the plants exposed to light were very short and bright green in appearance, while those from the darkroom were

TABLE 4  
EFFECT OF LIGHT (GREENHOUSE) AND AGE ON ASCORBIC CONTENT OF AVENA COLEOPTILES

AGE (HOURS AFTER SOAKING SEEDS)	IN LIGHT 36 HOURS	IN DARKROOM
72 . . . . .	0.750 mg./gm.	0.460 mg./gm.
96 . . . . .	0.430	0.354
172 . . . . .	0.327	0.314

only a faint yellowish green. It can be seen from these experiments that when plants contain chlorophyll and are exposed to light, ascorbic acid is formed in the coleoptile, accumulating in the tip more than in other parts. This may mean that the seat of synthesis is at the tip, as in the case of auxin. The precursor is probably mobilized from the seed reserves. That age decreases the ascorbic acid content is understandable on the basis that the seed reserves are exhausted during growth, and the precursor is depleted. Assimilation may replace this depletion, as seen by the preceding data (table 4).

The explanation for the decrease of the reduced and the increase of the oxidized ascorbic acid from tip to base of the coleoptile suggested a gradient of oxidation from tip to base, catalyzed possibly by an enzyme in the plant. This possibility was examined. The following protocol shows the result of one such experiment.

PROTOCOL.—Coleoptile sections from 100 seedlings were cut into tip, middle, and base sections, and weighed. They were macerated

in 3 per cent metaphosphoric acid, filtered, and the filtrates diluted with M/15  $\text{PO}_4$  buffer (at pH 6.0; 0.01 M cystine added to stabilize the ascorbic) in such a way that the inequality in weight of the sections was balanced by appropriate dilution. Ascorbic acid (Roche) was added to each of the three lots, and to the buffer control (1 cc. of a solution made by dissolving 0.0114 gm. ascorbic in 10 cc.). The time was recorded. One cc. of each lot was diluted with 5 cc. of 3 per cent metaphosphoric acid, and titrated at intervals with 0.001 M iodine solution. The results in table 5 are tabulated in cubic centimeters of iodine, and represent the relative amounts of ascorbic acid remaining in each solution.

TABLE 5  
GRADIENT OF ENZYMATIC DESTRUCTION OF ASCORBIC ACID IN  
AVENA COLEOPTILES (CC. IODINE)

TIME (MINUTES)	BUFFER	TIPS	MIDDLES	BASES
40 . . . . .	1.80	1.80	1.66	1.34
60 . . . . .	1.80	1.75	1.61	1.26
135 . . . . .	1.66	1.57	1.45	0.94
255 . . . . .	1.66	1.36	1.30	0.64

A similar experiment showed essentially the same result, as did a third. In the latter experiment, however, the buffer solution destroyed more ascorbic acid than did the plant tissue. Three experiments were run in which the pH was 6.6. No difference in destruction was found between apex and base. This may be explained by the fact that spontaneous oxidation of ascorbic acid occurs more readily at higher pH values, and could thus obscure small differences in destruction in the coleoptile. It is demonstrated by these experiments that the tips of coleoptiles oxidize less ascorbic acid than the bases, when the pH is adjusted to approximate that of the plant sap.

Since it is indicated in these experiments that the destruction of ascorbic acid is a function of the pH, it was thought possible that the gradient of destruction might be explained by a corresponding pH gradient in the plant. The evidence does not favor this concept, as BABIČKA (1) reported that exudates from apical cut sur-

faces of stems of several different plants are more acid than those from basal cut surfaces. BONNER moreover reported no such pH gradient in the *Avena* coleoptile. It is interesting that the pH of the plant sap (about 6.0) is just low enough to prevent rapid destruction of ascorbic acid. Experiments have shown that the ascorbic acid is not destroyed very rapidly in coleoptile extracts until the pH is higher than 6.0.

VAN OVERBEEK (7) has shown that heteroauxin is destroyed more by basal parts of coleoptiles than by apical parts, and that basal parts of mesocotyls contain more oxidative enzymes (peroxidase) than apical parts. Whether the gradient of oxidative enzyme for ascorbic destruction in the coleoptile bears any relation to such a peroxidase distribution remains to be shown.

The relation of ascorbic acid to plant growth is not yet known. It is known that ascorbic acid increases regeneration of willow branches, increases germination, and accelerates growth when supplied to the root system (3, 5, 6). Hence it was thought interesting to investigate the possible role of ascorbic acid as a growth hormone, or whether it acts together with a growth hormone such as auxin. The activity of ascorbic acid as a growth hormone was therefore tested, using the standard *Avena* technique (9). Ascorbic acid in agar blocks buffered at pH 5.0 to 6.0 had no effect on the curvatures, although it acted as an acid when unbuffered. In this case curvatures were produced, but since any other acid will presumably do this because of effects on the dissociation of regenerated auxin in the plant, it was concluded that ascorbic acid is not a cell elongation hormone like auxin. Ascorbic acid mixed with indole-3-acetic acid was likewise inactive in the *Avena* test as ordinarily performed. There is some possibility that it can act as a "food factor" in growth (9), since small curvatures were produced when auxin was made no longer limiting in its effect on growth. (This can be done by supplying high concentrations to the decapitated coleoptile for some time before applying the agar blocks containing the auxin to be tested.) Ascorbic acid in buffered solutions, stabilized with cystine, and with or without auxin, had no effect on the pea test for growth hormones (9). It was concluded that ascorbic acid must act in some other way on growth in plants than on the auxin mechanism concerned

in cell elongation. It is possible that the growth effects claimed for ascorbic acid are related to cell proliferation, as BONNER (unpublished) has found effects of ascorbic acid on the growth of pea embryos and buds in nutrient solutions.

### Summary

1. Ascorbic acid is present in considerable concentrations in *Avena* coleoptiles from etiolated seedlings.

2. On the basis of wet weight determinations, reduced ascorbic acid is present in higher concentrations in tips than in bases of coleoptiles, and oxidized ascorbic acid shows the reverse gradient of distribution.

3. The distribution of ascorbic acid in the coleoptile corresponds with the distribution of chlorophyll in plants grown in the light, but does not depend upon chlorophyll either for its presence or for its distribution in the coleoptiles of plants grown in the dark.

4. Light increases the concentration of ascorbic acid in the coleoptile, and aging decreases it.

5. Ascorbic acid is not present in the germinating seed, but is synthesized in the coleoptile from a precursor in the seed.

6. Reduced ascorbic acid is oxidized more by extracts from basal sections of coleoptiles than by extracts from apical sections. This destruction gradient corresponds with the destruction gradient of auxin in the coleoptile.

7. Ascorbic acid is not a cell elongation hormone like auxin, nor does it facilitate the action of auxin in the standard *Avena* or pea tests.

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# POLARIZED GROWTH AND CELL STUDIES IN THE FIRST INTERNODE AND COLEOPTILE OF *AVENA* IN RELATION TO LIGHT AND DARKNESS

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(WITH EIGHT FIGURES)

## Introduction

Much of the influence of light upon growth and development in plants has recently been explained by the role of growth hormones. Since rate of growth in *Avena* has been shown to be proportional to the concentration of growth hormone (21), within certain limits, differential distribution or inactivation of these growth regulating substances might be expected to modify the relative growth and development of the component parts of the seedling. Determinations of growth hormone in coleoptile tips of *Avena* seedlings have shown that the values obtained for irradiated plants are much lower than for plants maintained in darkness (7, 8, 17). Many differences in the external appearance of plants grown in light and darkness are well known and have been described at length in the literature. The question naturally arises as to the proportionate influence of light upon the cellular activities in growth; that is, cell multiplication and cell enlargement.

The nature of the first internode of the *Avena* seedling and the vascular relationship of its several parts have been described (3), and the gross form and microscopic structure of the coleoptile (grown in a darkroom under conditions favorable for its use as a standard test object for the quantitative determination of plant hormones) is now well known (1). Cell elongation and increase in cell number take place only in the plane of the long axis; that is, growth is polarized. The cell and tissue responses in the growth of the coleoptile exposed to light have not yet been investigated. Studies of phototropism of the coleoptile and growth of the first internode of *Avena* in relation to light have indicated marked differences in sensitivity and growth



response of the two organs. The coleoptile is very sensitive to the blue region of the spectrum, but not to the red (14); its growth in weak red light is about the same as in darkness. The first internode, however, attains considerable length when grown in complete absence of light, but its growth is appreciably shortened by exposure to light of all wave lengths. The influence of different dosages of light, as to quality and amount, upon the proportionate development of the coleoptile and internode, as well as the number and size of the cells in these organs, is unknown.

The purpose of this study was to investigate: (a) the influence of light and darkness upon growth of the coleoptile and first internode of different varieties of oats; (b) the influence of different intensities of light upon the first internode; (c) the influence of different intensities of light upon the coleoptile; and (d) the influence of light and darkness upon cell division and cell enlargement in the coleoptile and first internode throughout their ontogeny.

### Investigation

#### LENGTH OF FIRST INTERNODE AND COLEOPTILE OF DIFFERENT VARIETIES OF OATS

Size of the first internode and coleoptile of several varieties of oats was determined for seedlings grown (1) in complete darkness and (2) for a short time in light and then in darkness. The seeds, obtained through the courtesy of Mr. T. R. STANTON of the United States Department of Agriculture, Washington, D. C., were planted in moist clear quartz sand contained in glass vessels. One series was placed immediately in a darkroom and kept there until the first foliage leaves burst through the coleoptiles. The other series was subjected to daylight in a greenhouse during the first ten hours after planting and then placed in the darkroom. The darkroom temperature was 80° F. and the relative humidity 88-90 per cent. Measurements of the length of first internodes and length and diameters of coleoptiles are recorded in table 1.

The first internode failed to elongate to any extent in the series receiving a preliminary light exposure; in this same series also the embryonic foliage leaves burst through the coleoptiles about two days earlier than those of plants grown in complete darkness, the

latter group requiring about eight days. Decided differences in length of first internode and coleoptile were shown by the different varieties. Grown in complete darkness, most varieties possessed coleoptiles which were longer than the first internodes; a few varieties, such as Victory, Albion, and Hairy Culberson, gave the reverse relationship. All varieties exhibited great elongation of the first inter-

TABLE 1

SIZE OF FIRST INTERNODE AND COLEOPTILE IN DIFFERENT VARIETIES OF OATS  
SEEDLINGS GROWN IN DARKNESS WITH AND WITHOUT PRELIMINARY EX-  
POSURE TO DAYLIGHT; TEMPERATURE 80° F., RELATIVE HUMIDITY 90

VARIETY	COMPLETE DARKNESS				LIGHT EXPOSED		
	No. PLANTS MEASURED	FIRST INTERNODE LENGTH	COLEOP- TILE LENGTH	COLEOP- TILE DIAMETER	No. PLANTS MEASURED	COLEOP- TILE LENGTH	COLEOP- TILE DIAMETER
Black Tartar. . . .	20	30.7	53.8	1.5	23	38.2	1.6
Storm King. . . .	6	57.0	64.4	1.8	21	52.5	1.7
Black Mesdag. . . .	19	18.6	47.8	1.7	23	34.7	1.7
Victory. . . . .	13	47.2	44.2	1.5	20	40.7	1.5
Navarro. . . . .	23	22.4	55.6	1.7	22	44.4	1.6
Carleton. . . . .	22	46.0	66.7	1.5	22	49.8	1.6
Victoria. . . . .	23	42.8	62.7	1.6	22	47.5	1.4
Fulghum. . . . .	16	23.8	49.8	1.7	23	52.8	1.6
Richland. . . . .	15	41.6	50.2	1.5	24	43.5	1.5
Markton. . . . .	21	46.9	59.1	1.6	21	45.7	1.5
Coastblack. . . . .	21	40.0	55.6	1.6	22	55.5	1.6
Bond. . . . .	23	23.7	56.5	1.6	24	45.0	1.6
White Tartar. . . .	22	45.5	54.3	1.6	24	46.8	1.5
Albion. . . . .	18	51.3	48.6	1.5	24	42.7	1.5
Support. . . . .	23	35.0	64.2	1.6	15	48.4	1.6
Golden Giant. . . .	22	12.0	46.2	1.5	21	44.3	1.4
Hairy Culberson. . .	20	55.0	50.2	1.5	23	42.0	1.5
Aurora. . . . .	22	23.7	50.8	1.5	15	34.3	1.5
Average. . . . .		36.8	54.4	1.55		44.9	1.58

node in complete darkness, while exposure to bright light at the beginning of germination inhibited growth in this region almost completely. The average total length of the seedling shoot (first internode plus coleoptile) at the time the foliage leaves emerge is, in the completely darkened plants, more than twice as great as in those given preliminary illumination. The significance of this fact from the standpoint of survival of seeds germinating in darkness at relatively great depths in the soil is apparent. Those plants able to reach

light before exhaustion of the stored food supply would appear to have a decided advantage.

LIGHT INTENSITY AND LENGTH OF FIRST INTERNODE  
IN SEEDLINGS OF VICTORY OATS

In order to determine what relationship might exist between length of the first internode and intensity of the light under which they germinate, Victory oat seeds were placed upon wet coarse cheesecloth tied over glass vessels filled with distilled water (fig. 1).

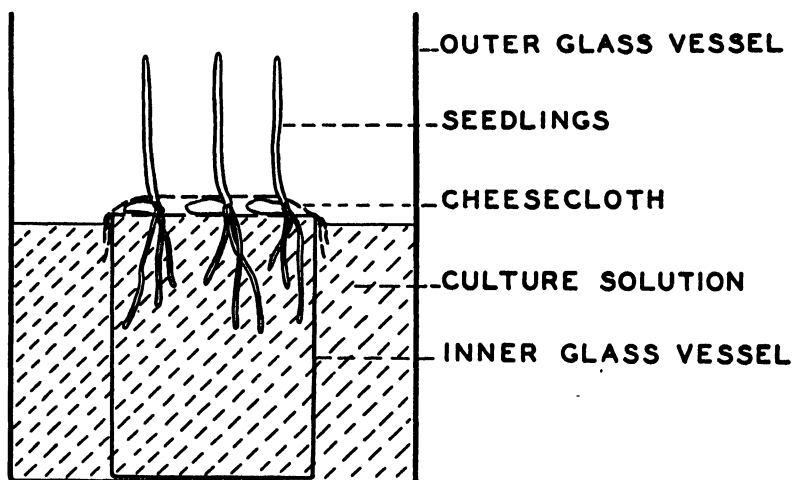


FIG. 1.—Culture vessels for growing seedlings: a small glass vessel filled with culture solution was placed inside a larger vessel containing the same solution and the level of the water in germinating vessel was maintained so that the seeds were kept well moistened. Seeds planted between two layers of coarse cheesecloth (held in place with a rubber band) grow well without further attention.

With this simple apparatus it was possible to place dry seeds upon the wet coarse cheesecloth surface and leave the seedlings to grow without further attention under constant conditions in a room maintained at 80° F. and 90 per cent relative humidity.

The first experiment was performed with a single 5 watt Neon glow lamp operated on a 110 a.c. line. The lamp was placed at one end of the darkroom and the seedlings germinated at different distances from the lamp. Further reduction in intensity was obtained by using neutral filters for the lowest two intensities. The light beam

was reflected vertically downward on to the seedlings by means of plane glass mirrors at an angle of  $45^\circ$ . Average measurements of first internodes of plants grown under different intensities and in darkness are given in table 2 and figure 2.

In table 2A the relative intensity is given when the highest value is set at 1; the maximum energy incident upon the plants was not accurately determined, but was approximately  $0.1 \text{ erg/mm.}^2/\text{sec.}$  Pronounced shortening of the first internodes was brought about by

TABLE 2  
FINAL LENGTH OF FIRST INTERNODE IN VICTORY OATS GROWN UNDER  
DIFFERENT INTENSITIES OF LIGHT; TEMPERATURE  $80^\circ \text{ F}$ ,  
RELATIVE HUMIDITY 90

A. LIGHT FROM 5 WATT NEON GLOW LAMP							
Relative intensity . . . . .	0	0.01	0.02	0.059	0.174	1 0	
Number of plants. . . . .	19	26	25	23	23	23	
Length of internode (mm.)	67.1	26.4	17.4	16.8	12.2	4.8	

B. LIGHT FROM 7.5 WATT MAZDA LAMP							
Intensity (ergs/mm. <sup>2</sup> /sec.)	0	0.027	0.154	0.224	0.585	2.006	3.363
Number of plants. . . . .	80	95	95	97	91	51	69
Length of internode (mm.)	71.2	40.4	24.6	18.7	12.7	8.34	2.20

comparatively small amounts of radiant energy in this region (red) of the spectrum.

A similar experiment was performed with a 7.5 watt Mazda lamp as the source of radiation. The energy was measured with an Eppley thermopile and a Leeds and Northrop type HS galvanometer. The energies and corresponding lengths of first internodes are given in table 2 and figure 3. An average first internode length of 71.2 mm. was attained in complete darkness. Marked shortening of the internode occurred under the lowest intensities used. The plotted data form a curve which shows pronounced effects of light at relatively low energy levels; comparatively little further shortening of the internode could be brought about by energy values greater than those plotted.

CELL NUMBER AND LENGTH OF COLEOPTILE IN VICTORY  
OATS IN RELATION TO INTENSITY OF LIGHT

Preliminary experiments were performed in the greenhouse to test the influence of daylight and darkness upon the final length attained

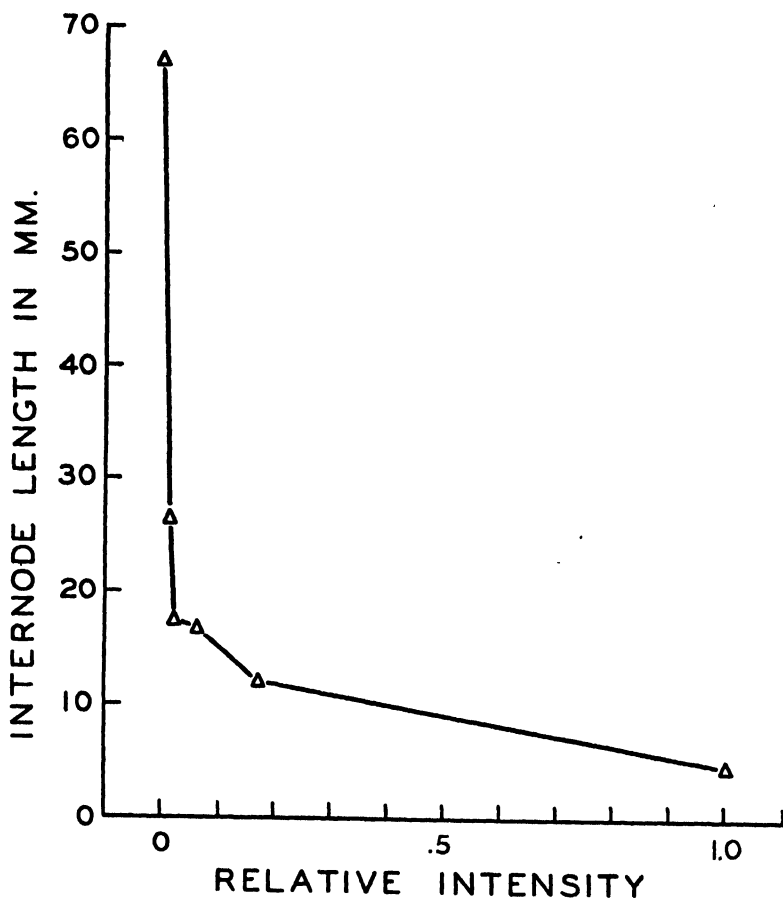


FIG. 2.—Length of first internode of *Avena* seedlings in relation to intensity of Neon light. An intensity of about 0.1 erg/mm.<sup>2</sup>/sec. was sufficient to shorten the internodes to one-fourteenth the length attained in darkness (see table 2).

by *Avena* coleoptiles. Seeds were planted in water culture vessels inclosed under bell jars. One set was placed in darkness and the other allowed to grow in moderately bright light. After about one week,

the coleoptiles were measured. The average final length of twenty-five illuminated coleoptiles was 18.7 mm. Another set of six plants grown under six 300 watt Mazda lamps (screened through a 5 cm. water cell) reached 24.7 mm. in length. Controls in darkness attained a length of 37.4 mm.

The final length attained by coleoptiles under different intensities of light was studied by growing seedlings in water cultures exposed

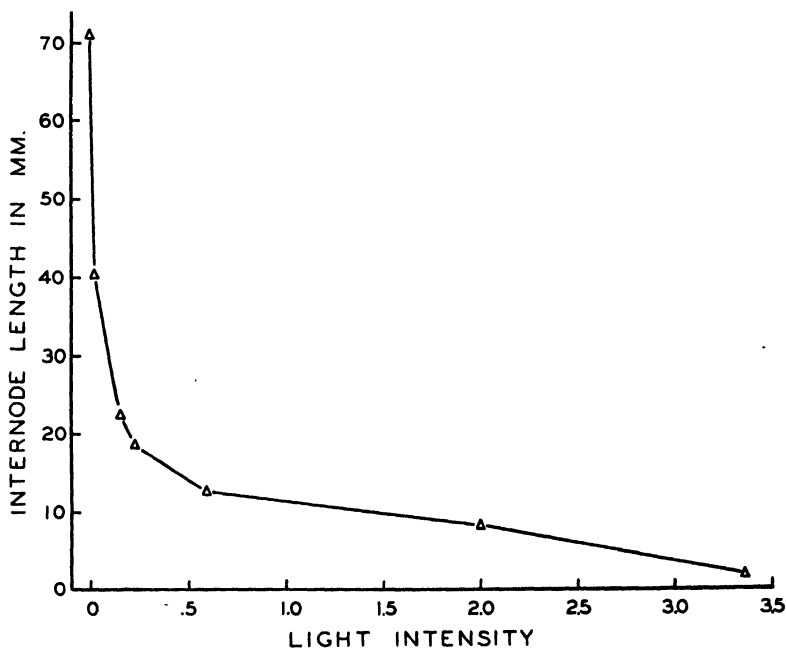


FIG. 3.—Final length attained by first internode of *Avena* in relation to intensity of Mazda light (see table 2). Intensities plotted as ergs/mm.<sup>2</sup>/sec.

to the light of a 6 inch mercury in quartz arc operated at 220 volts d.c. and 7.5 amperes. A plate glass water cell 5 cm. thick was placed between the arc and the plants for the purpose of filtering out the lethal ultraviolet and the infra-red radiation. Plants were grown in large metal boxes under seven different intensities obtained by varying the distance from the arc and by using black cloth screens. One set of plants was grown in darkness. The temperature about the plants was maintained at 80° F. in an air conditioned room.

After about four days the plants exposed to appreciable amounts of radiation had embryonic foliage leaves bursting through their coleoptiles, but those in the lowest intensities and in darkness required six or seven days for completion of coleoptile growth. All coleoptiles and internodes were measured for length, and then fixed in Craif solution (19) for cell studies.

The results of this study are presented in table 3. Marked shortening of the internodes was apparent under all intensities of light, but the coleoptiles were appreciably shortened only under

TABLE 3

FINAL LENGTH, AND CELL COUNTS IN COLEOPTILE (AND LENGTH OF FIRST INTERNODE) OF VICTORY OATS GROWN IN WATER CULTURE UNDER DIFFERENT INTENSITIES OF LIGHT FROM MERCURY IN QUARTZ ARC

Intensity (ergs/mm. <sup>2</sup> /sec.) . . . . .	0 07	0 14	0 42	1. 24	2 24	83 63
Length of coleoptile (mm.) . . . . .	50 4	49 4	49 9	45 1	40 1	23 7
Number of cells* (each figure the average of 4-8 coleoptiles) . . . . .	141	.	150	.	154	118
Length of first internode (mm.) . . . . .	13 0	3 3	1 3	0 3	0	0
Number of plants . . . . .	15	18	17	18	18	32

\* Unelongated cells at tip of coleoptile not included in these cell counts.

the highest intensity used. Note the greater inhibition of internode elongation by radiation from a mercury arc as compared with the same intensity of light from a Mazda lamp (tables 2 and 3).

#### CELL NUMBER AND SIZE IN FIRST INTERNODE OF VICTORY OATS IN RELATION TO INTENSITY OF LIGHT

A selected number of typical first internodes, obtained in the experiment dealing with the influence of low intensities of Mazda light on polarized growth in this region of the oat plant, were imbedded in paraffin, sectioned at 14  $\mu$ , and stained with crystal violet. Counts were made of the number of cells in the fourth layer of cortical parenchyma from the level of divergence of the epiblast to that of the coleoptile (figs. 4-6). The averaged measurements are recorded in table 4 and figure 7. It may readily be seen (table 4) that the number of cells varies inversely with the intensity of light. Calculated values for the length of the cells show a decrease with increasing light intensity. In the graph (fig. 7) the curves for internode length, cell number,

and cell size run more or less parallel throughout the entire intensity range. In the plants grown in complete darkness, the cell number increases slightly more rapidly than the internode length, and the average increase in cell size is not so great as the increase in internode length. Since internode length equals cell number times cell size, it seems to follow that in darkness multiplication of cells is favored and

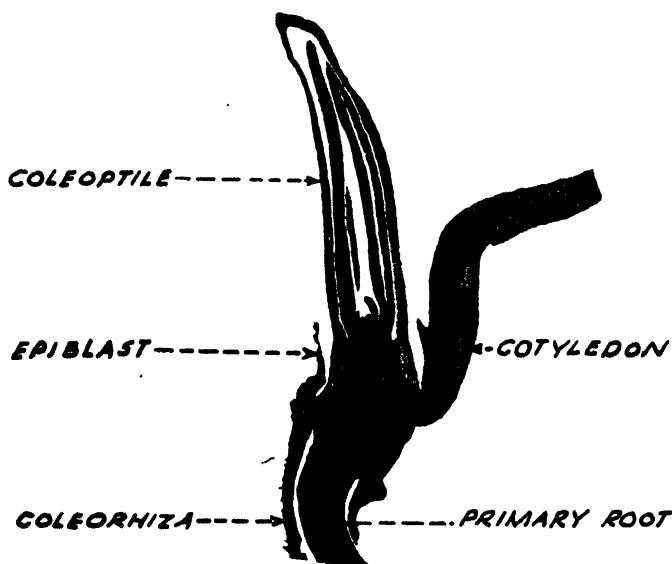


FIG. 4.—Median longitudinal section of *Avena* seedling, grown in light for 36 hours, endosperm, etc., removed (see table 5).

the length of the cells does not increase beyond a certain maximum. The question arises whether the maximum size attainable by cells before division is initiated may be controlled by light. Further evidence along this line is presented in the following.

#### CELL DIVISION AND ELONGATION DURING EARLY STAGES OF GERMINATION IN LIGHT AND DARKNESS

Seeds of Victory oats were planted in water culture vessels, one set being kept in a darkroom and another set under light supplied by a 1000 watt Mazda lamp and filtered through a 5 cm. thick water



cell. The temperature was controlled at 80° F. in each of the air conditioned rooms. At stated intervals (table 5) plants were picked

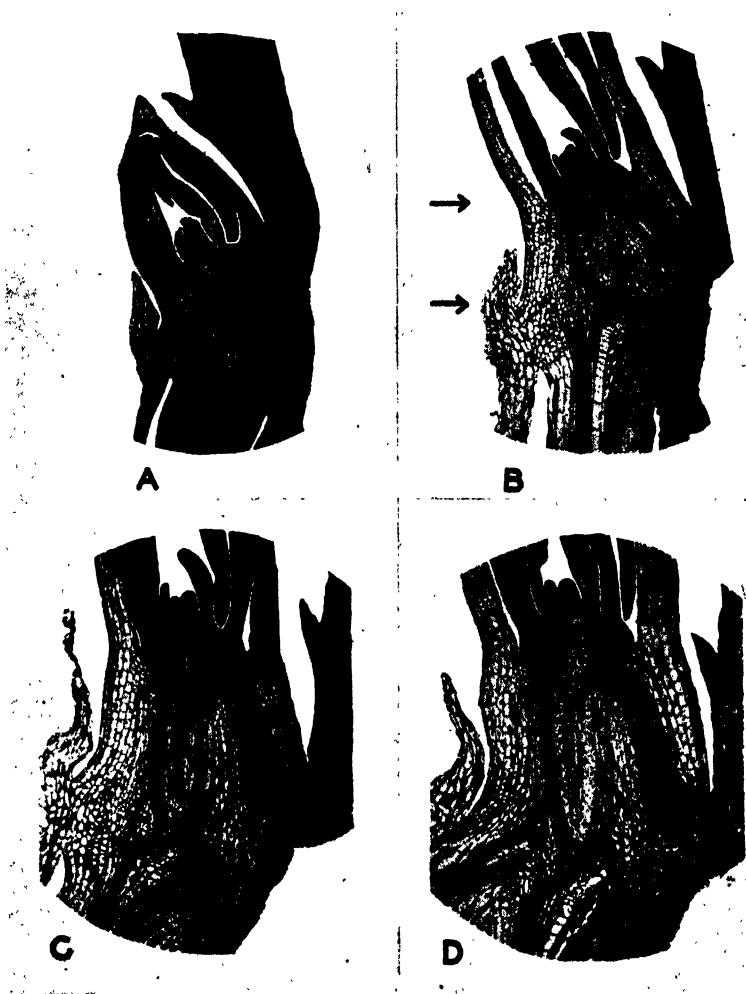


FIG. 5.—Median longitudinal sections of first internode, etc., of *Avena* seedlings, grown in light. Upper and lower limits of first internode indicated by arrows. A (embryo), after 6 hours; B, after 24 hours; C, after 36 hours; D, after 42 hours (see table 5).

in Crai solution and later imbedded in paraffin, sectioned, and stained with crystal violet. The lengths of the internodes and coleop-

tiles were measured on the slides, and the number of cells in the fourth row of the cortex in the first internode or median row in the coleoptile was counted. From these data the average size of cells was

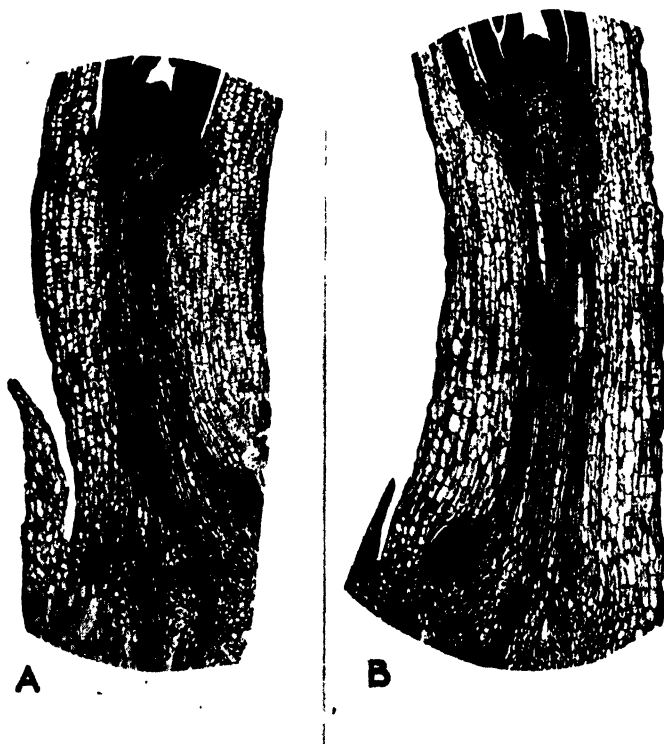


FIG. 6.—Longisections of first internode of *Avena* seedlings, grown in darkness. *A*, after 36 hours; *B*, after 42 hours (see table 5). In *B*, particularly, note the meristematic region (with smaller cells) in the upper portion of the internode. Whenever light conditions are such that the first internode elongates, it is at first meristematic throughout. As it grows longer, the meristematic region becomes localized in the upper end of the internode, in the zone just below the coleoptilar node.

computed. Five or six plants were used for determining each average figure presented in table 5 and figure 8. See also figures 4–6.

The data indicate a very small amount of polarized growth in the internodes of plants grown in light. Microscopic evidence indicates that no cell division and only moderate enlargement of the cells took

place. In contrast, the internodes kept in darkness elongated slightly during the first thirty-six hours after initial soaking in water, and thenceforth grew rapidly until a length of 46.4 mm. was attained

TABLE 4  
FINAL CELL NUMBER AND SIZE IN FIRST INTERNODE OF VICTORY OATS  
GROWN IN WATER CULTURE UNDER DIFFERENT INTENSITIES  
OF MAZDA LIGHT

Intensity (ergs/mm. <sup>2</sup> /sec.)	Dark	0.154	0.585	2.006	3.363
Number of cells	159.0	57.7	47.3	34.5	25.0
Length of internode in mm. (on slides)	72.8	27.0	17.9	10.6	4.3
Computed length of cells ( $\mu$ )	458	468	378	307	172
Number of plants	8	9	9	11	7

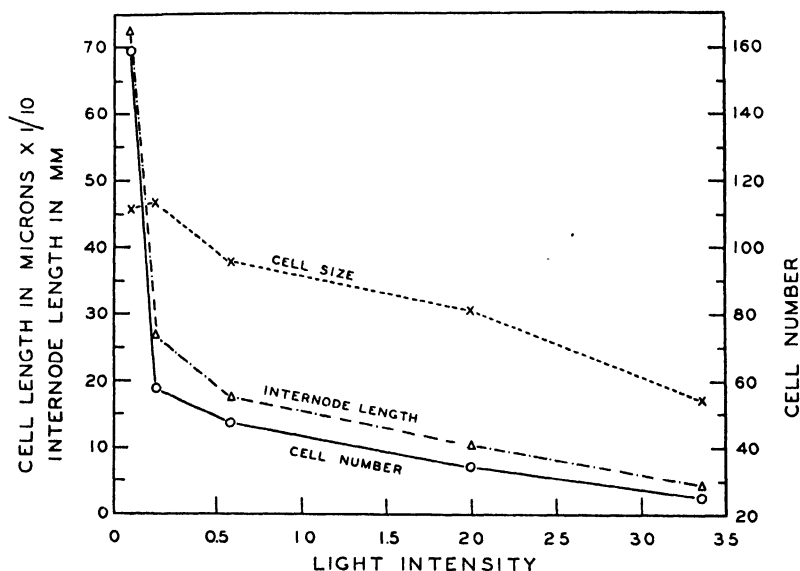


FIG. 7.—Cell number and cell size in first internode of *Avena* in relation to light intensity. Increased length of internode in progressively lower light intensities accounted for by increased numbers of cells as well as larger cell size; in complete darkness, however, the considerable further elongation of internode is due to larger number of cells resulting from further meristematic activity.

after eighty-six hours. Cell number increased a little faster than length of internode, hence cell length did not increase directly in proportion to length of internode in the dark-grown seedlings.

TABLE 5  
LENGTH, CELL NUMBER, AND CELL SIZE OF FIRST INTERNODES AND  
COLEOPTILES OF AVENA SEEDLINGS AT DIFFERENT  
AGES IN LIGHT AND DARKNESS

	AGE OF SEEDLING											
	6 HOURS		24 HOURS		36 HOURS		42 HOURS		86 HOURS		206 HOURS	
	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK
Internode cell number	19 5	18.6	19 8	20 5	19 0	31.5	22	42 2	19 8	144		159
Internode length (mm.)	0 264	0 283	0 493	0 339	0 683	0 813	0 864	2 66	1 2	46 4		72 8
Computed cell length ( $\mu$ )	13 5	15 2	24 9	16 5	35 9	26 2	39 3	63 0	60 8	322		458
Coleoptile cell no.	45	45	83	61	114	108	142	162				...
Coleoptile length (mm.)	0 831	0 787	1 836	1 228	3 654	3 657	7 45	5 746				...
Coleoptile, computed cell size ( $\mu$ )	18 2	17 5	22 0	20 0	32 1	33 9	52 3	35 5				....

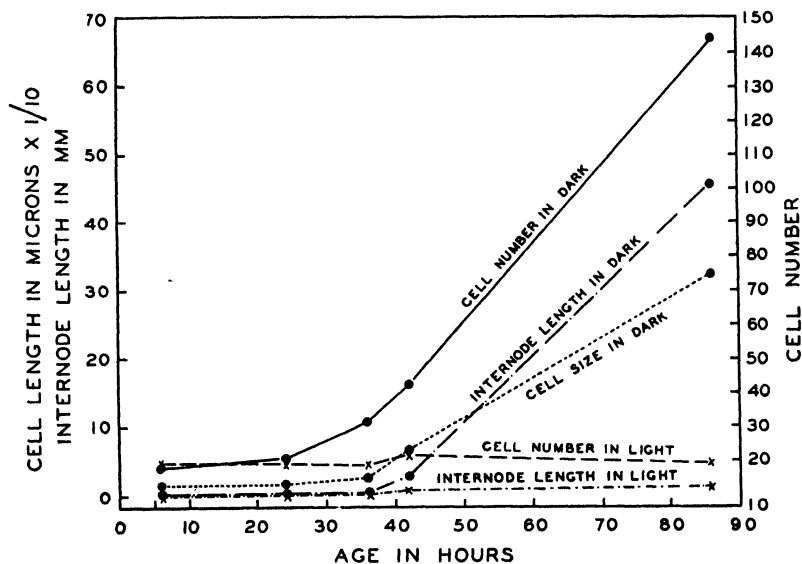


FIG. 8.—Length of first internode in *Avena* and number and size of its constituent cells at different stages during germination in light and darkness. Note that greater internode length in plants grown in darkness is due to increase in number as well as in size of cells. In contrast, internodes of plants grown in strong light (four feet from 1000 watt Mazda bulb) do not elongate; their cells fail to divide, and elongate only slightly. See table 5 for cell behavior in coleoptiles of same seedlings.

The data obtained in a study of cell behavior in the light and dark-grown coleoptiles are shown also in table 5. In both the light and dark-grown plants, the coleoptile cell numbers increased markedly during the first twenty-four hours and the number had more than trebled at the end of forty-two hours. The Mazda illumination had comparatively little influence upon length of the coleoptile and behavior of its constituent cells in the early stages of germination. It seems to be a significant fact that light of low or moderate intensity inhibits growth of the internode, but at the same time exerts but little influence upon the coleoptile. The effect of very intense light upon early aging of the coleoptile has already been brought out.

### Discussion

The first internode of grass seedlings grown in darkness undergoes marked elongation (polarized growth). Numerous factors have been suggested as the cause of this elongation, among them low temperature, low moisture content of the soil, and high CO<sub>2</sub> content of the atmosphere (4). Many years ago ROTHERT (20) pointed out that temporary illumination was effective in checking the development of this internode. Through the investigations of LANGE (15, 16), BEYER (2), DU BUY and NUERNBERGK (9, 10, 11), and HAMADA (12, 13), it has become clear that elongation of the *Avena* first internode may be suppressed by irradiation even for brief periods and with both long and short wavelengths of light. LANGE (15) pointed out that phototropically inactive red light, applied during the period of swelling and for twelve to sixteen hours after emergence of the coleoptile, inhibited elongation of the internode. HAMADA worked with the effect of light and temperature in relation to age of the seedling as factors concerned in internode shortening. The age at which maximum inhibition was brought about by light came earlier in germination, the higher the temperature. DU BUY (8) found that monochromatic blue and red light was very effective in shortening both internode and coleoptile. Elongation can be checked also by heat radiation (10). VAN OVERBEEK (17) has reported that exposure to the red plus infra-red radiation of an ordinary darkroom lamp brought about short internodes and decreased growth hormone con-

tent in the tips of *Avena*. It is known from the work of several investigators (4, 7, 8, 21) that high intensities of radiation inactivate plant growth hormone; also that the hormone is a limiting factor in extension of the internode under conditions of exposure to light.

JOHNSTON (14) has found that internode elongation can be inhibited by exposure to extremely low intensities of red and blue light in which the plants are invisible to the human eye. The data reported here indicate that the effective radiant energy required for threshold inhibition is very low. Less than one erg/mm.<sup>2</sup>/sec. was sufficient to bring about an appreciable effect, so it may be seen that the mechanism involved is extremely photosensitive.

DU BUY and NUERNBERGK (11) studied the number of cells in longitudinal sections of first internodes shortened by light and found that no cell division took place under the conditions of their experiments. The experiments reported here with different intensities of light show that the extent of cell division in the internode depends upon the amount of light. Below the intensity causing maximum inhibition, cell multiplication takes place to an extent which varies inversely with the intensity and almost directly with the final length of internode attained.

The gain in length of the first internode (grown in darkness) may be found from the data of table 5, where the average size in the embryo was 0.283 mm. and the final length 72.8 mm. The final length was 257 times the original length in the seed. Analysis of the cell behavior shows 8.5 times as many cells in the mature internode and an average cell length of 30.1 times that in the embryo. Further analysis shows that in strongly illuminated internodes the cells increase in average length to about 4.5 times their original size, or from 13.5 to 60.8  $\mu$ . The striking difference between cell behavior in darkness and light is in the cell division activity. In the light no division takes place, and hence the internodes remain at about the same length as that attained by the dark internodes in the swelling stage just prior to initiation of their cell divisions.

In a study of the influence of light and darkness upon cell behavior in the epicotyl of *Phaseolus multiflorus*, BROTHERTON and BARTLETT (5) concluded that light "retards extension of the cells, and that as

an indirect result there are fewer secondary divisions, since relatively fewer primary cells enter the range of length within which division takes place." PENFOUND (18) observed that the increased height of shade-grown *Helianthus* seedlings was accompanied by an increase in the number of cells, not increase in length of cells, in the longitudinal axis. A number of other investigators have found that the effect of light upon growth of organs and tissues is concerned with both cell division and cell extension (*cf.* BURKHOLDER, 6).

Examination of longitudinal sections through *Avena* embryos in early stages of germination in both light and darkness has shown that during the uptake of water considerable swelling occurs, accompanied by enlargement of the cells. In strong light the cells of the internode reach an average length of  $35.9\ \mu$  at the end of thirty-six hours. At this time the cells of the darkened plants are beginning to divide; their average size is  $26.2\ \mu$ . Measurements of cells which had been in division stages in darkness yielded length values of 28 and  $36\ \mu$  at thirty hours of age.

The cells of illuminated internodes increase in size just as do those in darkness during early germination, but they do not divide. Their further enlargement is negligible; the internode fails to grow. It is clear that in early seedling growth light inhibits cell division in the internode; this is not an indirect influence brought about through failure of the cells to enlarge. The influencing factors are probably concerned with certain substances necessary for cell division, and if so, such substances must be rendered ineffective, or are changed in their path of movement, by very low intensities of light.

### Summary

1. Twenty varieties of three species of *Avena* were germinated in complete darkness and with preliminary light treatment in the early stages of soaking, followed by growth in darkness. The ratio of internode length to coleoptile length differed markedly in the different varieties.

2. Final length of the first internode of *Avena sativa* var. Victory grown under a series of different intensities of weak Mazda light

varied inversely with the intensity. Similar inhibition of internode elongation in proportion to the intensity of Neon light was found at intensities below 0.1 erg/mm.<sup>2</sup>/sec.

3. Early stages of germination in Victory oats in light (1000 watt Mazda) and in darkness showed that the internode elongated slightly during early swelling (up to thirty-six hours) in both light and darkness. In darkness further polarized growth occurred in the internode, but in strong light growth ceased early and shifted to the coleoptile.

4. Different amounts of light influenced polarized growth in different organs and tissues in different ways. Very low intensities of light inhibited growth of the first internode but not that of the coleoptile; high intensities inhibited the internode and appreciably shortened the coleoptile (by decreasing the number of cells as well as cell length). In complete absence of light the internode grew extensively, and the coleoptile was somewhat shorter than in plants which received small amounts of light in the early stages of germination.

5. Analyses of cell behavior in the first internode when grown to maturity under different intensities of Mazda light indicated that both cell division and cell enlargement were responsible for polarized growth.

6. During early germination, cell enlargement (up to a certain size) was found to occur in the first internode whether the seedlings were grown in light or darkness; in later development, cell division occurred in darkness and under very low intensities but not in bright light. The size of dividing cells during early germination in darkness was somewhat less than that attained by the cells of internodes grown in light.

7. In darkness, both cell division and cell elongation contributed to growth of the internode in length. The number of internode cells increased from embryo to maturity by 8.5 times and the average cell length increased by 30 times.

8. The effect of light in shortening the first internode of the axis was brought about primarily by inhibition of cell division. It is suggested that the influencing factors are probably concerned with cer-



tain substances necessary for cell division, and if so, such substances must be rendered ineffective, or are changed in their path of movement, by very low intensities of light.

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# CULTIVATION IN VITRO OF EXCISED PEA ROOTS

JAMES BONNER AND FRED ADDICOTT

(WITH THREE FIGURES)

## Introduction

There are many complicated interactions between the individual parts of the developing organism. That these interactions are due, at least in part, to the production, transport, and action of particular chemical substances has perhaps been earlier only an hypothesis. The work of the past few years has shown, however, that some of the effects of one plant organ upon the development of another are to be ascribed to the substance auxin, a typical hormone, which is produced in particular regions of the plant and influences various processes, notably cell enlargement, in other regions. Auxin is not the only specific substance or factor involved in the process of development. In fact, auxin acts only in conjunction with other, as yet unknown, factors in the initiation of roots, the formation of swellings, etc. (25). It was with the hope of isolating and identifying still other substances concerned with growth and differentiation that the present work was undertaken.

HABERLANDT (9) was the first to suggest that the reciprocal relationships of cells and organs might well be studied through observation of the results obtained by severance of these relationships; that is, by cultivation of isolated cells and organs in vitro. The present paper is concerned with a problem of this kind, namely, the cultivation in vitro of plant roots.

The first relatively successful cultures of isolated root meristems were those of ROBBINS (17, 18), ROBBINS and MANEVAL (19), and KOTTE (12, 13). These workers cultivated tips of *Pisum* and of *Zea* roots in various media and established the important fact that an excised root meristem, if it grows, continues to develop as a normal root. Differentiation into the usual root tissues takes place and secondaries may even be formed. Moreover, both KOTTE and ROBBINS found that addition of extracts to the culture medium may some-

what improve the growth of isolated roots. ROBBINS and ROBBINS and MANEVAL used yeast autolyzate for this purpose. KOTTE obtained beneficial effects with meat extract. ROBBINS also attempted to subculture corn roots. Short tips were cut from the cultured root after it had grown to several times its original length, and these fresh tips were placed in fresh medium and allowed to grow again. This procedure was then repeated through several transfers or passages, and it was found that the growth rate decreased rapidly in successive transfers and that the roots eventually died.

WHITE (26, 27, 28) undertook an extensive series of investigations into the optimum conditions for the cultivation of excised roots. In 1934 he was able to announce the "potentially unlimited growth" of tomato roots in vitro (29). Tomato roots, transferred weekly and having an average growth rate of 6.2 mm. per day, were kept in culture for four years (32). The medium which WHITE used contained in addition to inorganic salts and sucrose 0.01 per cent of yeast extract. Since the yeast extract is essential for the growth of these roots, it must contain accessory growth factors which the root is unable to synthesize for itself.

Studies of the cultivation of roots in vitro have also been made by GAUTHERET (7), DAUPHINÉ (3, 4), CHAMBERS (2), GEIGER-HUBER and BURLET (8), LOO and LOO (14), FIEDLER (6), and others.

### Material and methods

Roots of *Pisum sativum* were employed in all the cultures. Attempts to culture these roots have previously been made by KOTTE (12, 13), ROBBINS, BARTLEY, and WHITE (20), and FIEDLER (6), but in no case have strikingly good results been obtained, nor has an extensive investigation of cultural conditions been made. The present work therefore began with a systematic inquiry as to the influence of several environmental factors and a search for a suitable culture medium.

Seeds of an inbred strain of peas, Perfection, kindly supplied by the Ferry-Morse Seed Company, were used for all of the later experiments. For the earlier tests seeds of Alaska and Perfection strains of the Moscow (Idaho) Seed Company were employed. The seeds were washed briefly with 95 per cent alcohol to remove a por-

tion of the fatty covering of the seed coat, sterilized in 0.1 per cent  $\text{HgCl}_2$  for twenty minutes, washed with sterile water, transferred to sterile petri dishes containing a small amount of sterile water, and allowed to germinate for forty-eight hours. At the end of this time the roots were 5–10 mm. long. A portion of the apical meristem (including the root cap) 3–4 mm. long was then excised with a surgical scalpel (Bard-Parker, no. 7 handle with no. 10 blade). Such knives were found to be more satisfactory and more convenient than scissors, fragments of razor blades, or ground sewing needles (20), and less expensive than cataract knives. Transfer of the root tips was accomplished with a fine pair of forceps, great care being taken to avoid injury to the meristems.

All cultures were carried out in a small room built for the purpose. The walls, table, and floor of this room were washed periodically with a dilute solution of Lysol. Immediately before the room was used it was washed out with a fine water spray which thoroughly removed bacteria and mold spores from the atmosphere. The culture table was then covered with a freshly sterilized cloth. Knives and forceps were sterilized in alcohol and flamed before using. Infections occurring during the culturing were extremely rare.

The cultures were kept in a darkened cabinet at room temperature. This entailed regrettable but unavoidable fluctuations of temperature with corresponding fluctuations in the growth rate of the roots, even though an attempt was made to keep the room as close to  $23^{\circ}$ – $25^{\circ}$  C. as possible. Table 1 gives the results of one experiment in which roots were cultured at three different temperatures under otherwise similar conditions, and shows the magnitude of the variations to be expected from this source.

A comparison of various methods of dishwashing was also made. It was found completely satisfactory to wash the culture vessels in sulphuric acid-dichromate cleaning fluid, followed by rinsing in tap and distilled water. This method gave better growth of the roots than washing the vessels with soap, and was not improved by other modifications such as additional rinsing with hot water.

In early cultures the growth increments were small (5–9 mm. per week) and the roots appeared definitely unhealthy, as judged by the frequent brown discoloration. This was due primarily to the fact

that ordinary laboratory distilled water had been used in making up the medium. Only when water redistilled from pyrex was used could regular and extensive growth be obtained.

Before a standard culture technique was finally decided upon, various types of culture vessels were investigated, namely 50 and 125 cc. Erlenmeyer flasks and 10 cm. petri dishes. When liquid medium was used in the flasks growth was irregular and extremely poor, in marked contrast to the behavior of tomato roots which grow excellently under such conditions (29). Agar medium in flasks was somewhat more satisfactory, but the best results were obtained with petri dishes. In these vessels root growth was as good on liquid as

TABLE 1  
EFFECT OF TEMPERATURE ON GROWTH  
OF EXCISED PEA ROOTS

TEMPERATURE	GROWTH IN MM. IN ONE WEEK
17° C.	18.2 ± 1.0
24	26.4 ± 1.8
27	29.3 ± 2.2

on agar medium. FIEDLER (6) has recently demonstrated the importance of adequate aeration for the growth of excised roots, and it seems probable that the success of the petri dish as a culture vessel is due to the fact that the medium is spread out in a relatively thin, well aerated layer. Ten cm. petri dishes, charged with 15 cc. of the medium under investigation, were therefore adopted as the standard culture vessels for the further experiments. Two roots were grown in each dish.

### Investigation

#### I. EXPERIMENTS ON FRESHLY EXCISED ROOTS

In any work of this kind, in which the cultural conditions are of great importance, the optimal conditions can be arrived at only by a series of successive approximations. For example, the iron concentration in the medium may be improved until some other factor becomes limiting. This second condition or factor may then be improved and further work upon the first factor thus made possible.

The present investigation was necessarily conducted in this way. For the sake of clearness, however, the results are not presented chronologically; so far as possible the experiments upon each culture condition are grouped together.

**EFFECT OF LENGTH OF INITIAL TIP.**—In order to ascertain the most favorable size of the initial fragment, tips of different lengths were cultured in the medium whose composition is shown in table 3. Table 2 gives the result of one such experiment. Tips having a length of less than 0.5 mm. very rarely developed into normal roots. In most cases the fragment either elongated slightly and then ceased

TABLE 2  
EFFECT OF LENGTH OF INITIAL ROOT TIP UPON  
GROWTH OF EXCISED PEA ROOTS

LENGTH OF TIP (MM.)	PERCENTAGE OF TIPS WHICH GREW AS NORMAL ROOTS	AVERAGE LENGTH OF NORMAL ROOTS
0.5 . . . . .	0	.....
1.0 . . . . .	18	7.4
2.0 . . . . .	50	25.8
3.0 . . . . .	100	44.0
4.0 . . . . .	100	44.3

growth completely, or developed into a colony of loose parenchymatous cells similar to those described by ROBBINS, BARTLEY, and WHITE (20) for very short corn root tips. In neither case was any trace of vascular tissue formed. Tips 0.5–1.0 mm. in length developed into normal roots in about 20 per cent of the cases. The growth rate of roots from these short fragments, however, was considerably slower than that of roots taken from longer ones. If tips greater than 3 mm. were taken, all of them grew as normal roots and their growth rate was unaffected by further increase of the initial length. Measurements upon sectioned roots similar to those used in the cultures showed that the distance from the apical end to the common initial zone is about 0.45 mm. The initial zone itself is less than 0.1 mm. in length. It is therefore clear that for normal growth of the root a considerable portion in addition to the initial zone is necessary. In particular, the development of vascular tissue must depend upon

the presence in the tip of well defined prospective vascular tissue, since such tissue was not formed by tips containing only the initial zone. The presence of the differentiating histogen zone alone was not sufficient for normal growth, however. If a 0.5 mm. fragment was removed from the root, normal growth of the following portion did not take place even if this portion was as long as 2 mm. Such fragments merely elongated for a brief period and then stopped growth completely. For the production of a normal root both the initial zone and a relatively large amount of the following portion are necessary. For all of the subsequent cultures tips having a length of 3-4 mm. were therefore used.

TABLE 3  
INORGANIC CONSTITUENTS OF MEDIUM USED FOR  
CULTIVATION OF EXCISED PEA ROOTS

SUBSTANCE	WHITE'S MEDIUM MG./LITER	PEA ROOT MEDIUM MG./LITER
$\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O} \dots$	142	236
$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O} \dots$	74	36
$\text{KNO}_3 \dots$	81	81
$\text{KCl} \dots$	65	65
$\text{KH}_2\text{PO}_4 \dots$	12	12
$\text{Fe}_2(\text{SO}_4)_3$ or. ....	2.5	2
Ferric tartrate. ....	.....	1

INORGANIC CONSTITUENTS OF MEDIUM.—For the first experiments, which were directed toward the satisfactory culture of freshly excised roots, a medium was chosen having the composition of that used by WHITE (29) for the culture of tomato roots. The composition of this medium is given in table 3. Two per cent glucose was used as a carbohydrate source. In this medium the freshly excised roots grew regularly 25-30 mm. during the first week. Additions of yeast extract, peptone, boron, manganese, and zinc, each over a wide range of concentrations, as well as variation of the glucose concentration, had little effect.

Growth was clearly limited by some factor or condition other than those investigated. There were of course a great number of possibilities. WHITE (28) has stressed the importance of the correct ionic composition of the medium, and it was therefore decided to investi-



gate the effects of varying the concentration of each salt. The roots were grown for one week under the standard cultural conditions just outlined. A medium having the composition shown in table 3 was used as a basis for the variations, since, as will be shown, this medium is on the whole the best of those tried. In each series one of the salts

TABLE 4  
INFLUENCE OF IONIC MILIEU ON GROWTH OF EXCISED PEA  
ROOTS IN 2 PER CENT GLUCOSE MEDIUM

RELATIVE CONCENTRATION OF SALT	GROWTH IN MM. IN ONE WEEK		
	Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	MgSO <sub>4</sub> · 7H <sub>2</sub> O	KNO <sub>3</sub>
	1X = 236 mg./l.	1X = 36 mg./l.	1X = 81 mg./l.
4X.....	22.3 ± 1.8	22.4 ± 1.1	20.2 ± 1.4
2.....	19.0 ± 2.4	24.0 ± 0.9	19.5 ± 2.2
1.....	30.0 ± 1.2	25.5 ± 0.9	23.5 ± 0.6
0.5.....	21.8 ± 1.5	24.6 ± 1.0	22.9 ± 1.0
0.25X.....	22.7 ± 1.6	24.6 ± 1.2	21.5 ± 1.7

RELATIVE CONCENTRATION OF SALT	GROWTH IN MM. IN ONE WEEK		
	KCl	KH <sub>2</sub> PO <sub>4</sub>	NH <sub>4</sub> NO <sub>3</sub>
	1X = 65 mg./l.	1X = 12 mg./l.	1X = 1250 mg./l.
4X.....	23.7 ± 1.1	32.9 ± 1.6	23.0 ± 1.2
2.....	27.2 ± 0.9	28.5 ± 1.0	27.5 ± 1.2
1.....	28.5 ± 1.5	30.6 ± 1.5	31.1 ± 1.5
0.5.....	27.8 ± 0.7	27.5 ± 1.4	23.2 ± 1.4
0.25X.....	23.3 ± 1.1	28.4 ± 1.4	27.0 ± 1.9

was used in concentrations of 4, 2, 1, 0.5, and 0.25 of its concentration in the basic medium. The results from a number of experiments are presented in table 4, together with the probable errors of each determination. These probable errors will suffice to give an indication of the variation to be expected when ten to twelve roots are used. Included in table 4 also is a series in which NH<sub>4</sub>NO<sub>3</sub> replaced the KNO<sub>3</sub>. It is evident that within the concentration limits considered,

the inorganic composition of the medium has little effect upon the growth of these roots. The substitution of ammonium for nitrate as a source of nitrogen is also without beneficial effect. One can conclude only that other factors are limiting the growth.

Various concentrations of iron in the form of  $\text{Fe}_2(\text{SO}_4)_3$  were used (table 5). Concentrations of 4 and 5 mg. per liter were markedly toxic, but the lower concentrations all appeared to have about the

TABLE 5  
INFLUENCE OF IRON CONCENTRATION UPON GROWTH  
OF EXCISED PEA ROOTS

$\text{Fe}_2(\text{SO}_4)_3$ mg./liter . . . . .	0.25	0.5	1.0	2.0	4.0	5.0
Growth in mm. in one week. . . . .	27	26	28	27	19	8

TABLE 6  
COMPARISON OF TARTRATE AND SULPHATE AS IRON  
SOURCE FOR GROWTH OF EXCISED PEA ROOTS

	GROWTH IN MM. IN ONE WEEK		
	1 mg./liter	2 mg./liter	3 mg./liter
Ferric sulphate. . . . .	26	26	27
Ferric tartrate. . . . .	27	26	25

same effect. It was possible that sources of iron other than the sulphate might be more favorable. Ferric chloride, ferric phosphate, ferric sulphate, and ferrous and ferric tartrate were therefore compared. It was found that when glucose was used as the carbohydrate source the tartrate was markedly more favorable. This difference disappeared, as is shown in table 6, when conditions were improved by the use of sucrose as the source of carbohydrate, and need not be gone into in detail.

A second series of experiments was run in which 2 per cent sucrose rather than 2 per cent glucose was used as the carbohydrate and in which the inorganic constituents of the medium were varied as in table 4. As is shown later, sucrose is superior to glucose for the pea root. In this series the variations of growth increment with changes

in the ionic composition of the medium were even smaller than in the first series. It is clear that under the conditions thus far examined the relative amounts of the various inorganic constituents of the medium are not of great significance in determining the growth of excised pea roots. The medium whose composition is given in table 3 is on the whole somewhat superior to those of the other compositions tried, and it was therefore used in all of the later experiments. It may well be, however, that when still other limiting factors are removed (see later) the ionic nature of the medium is of more importance.

TYPE OF CARBOHYDRATE.—There had been some indications that different sugars differed in their capacity to support the growth of pea roots. This is not surprising, since ROBBINS and WHITE (22) have shown that for *Zea* roots dextrose is superior to fructose, xylose, sucrose, or maltose. On the other hand WHITE (30) has found that sucrose is superior to glucose for tomato roots. A comparison between a number of sugars was therefore made. Baker c.p. glucose, Merck c.p. glucose, Pfanstiehl c.p. glucose (for injection), Pfanstiehl c.p. fructose, Pfanstiehl c.p. galatose, and Baker c.p. sucrose were used, each in 2 per cent concentration. The results of one experiment are shown in table 7. Galactose is used little if at all and the growth is slight. The three glucose preparations are much better and are essentially equal among themselves. Fructose is slightly better than glucose, but the Baker sucrose is definitely the best of all. This difference between glucose and sucrose as a carbohydrate source for pea roots has been found consistently in many experiments, but the reason for it is still obscure. It may be that the readily crystallizable sucrose contains less of some heavy metal impurity than does the glucose, or there may be some metabolic basis for the difference. It is of interest that pea roots resemble tomato roots in that they prefer sucrose to glucose, whereas the two grass roots which have been studied extensively (wheat and corn) definitely prefer glucose to sucrose.

CONCENTRATION OF CARBOHYDRATE.—The use of 2 per cent sugar has apparently become traditional among those who attempt the cultivation of excised roots. ROBBINS and MANEVAL (19), however, pointed out many years ago that growth in length, increase of dry

weight, and number of secondaries produced by excised *Zea* roots is greater in 4 per cent glucose medium than in 2 per cent. MALYSCHEV (15) apparently used sugars in concentrations other than 2 per cent, but his findings are not reported in detail. WHITE has used 2 per cent sugar, as have GAUTHERET (7), ROBBINS and coworkers (20, 21, 22, 23), LOO and LOO (14), FIEDLER (6), and others. There have been occasional brief departures from the tradition (13, 8), but the more extensive investigations have in general employed sugar in the concentration of 2 per cent. At the beginning of the present work different glucose concentrations were compared, but

TABLE 7  
COMPARISON OF VARIOUS SUGARS AS CARBOHYDRATE SOURCE FOR GROWTH OF EXCISED PEA ROOTS; EACH SUGAR USED IN 2 PER CENT CONCENTRATION

SUGAR	GROWTH IN MM. IN ONE WEEK
Glucose (Merck) . . . . .	22.5
Glucose (Pfanstiehl) . . . . .	22.0
Glucose (Baker) . . . . .	22.3
Fructose (Pfanstiehl) . . . . .	25.5
Galactose (Pfanstiehl) . . . . .	5.3
Sucrose (Baker) . . . . .	33.0

because of the small and variable growth of these early cultures, of course due to the fact that other conditions were at that time limiting, no obvious effect was observed. Different concentrations of sucrose were therefore tried, using the improved medium. A series of typical results is presented in table 8. In the absence of sugar the tips grew very little, and what growth did take place was undoubtedly to be attributed to sugar stored in the initial fragment. With increasing sucrose concentration the growth increment increased rapidly until 4 per cent was reached. Further increases were then without effect. It is clear that under the conditions used in these experiments growth is limited by sugar concentration unless the latter is 4 per cent or more. The fact that the earlier sucrose cultures were limited by the low sugar concentration may partially explain their lack of response to changes in the ionic milieu and to special factors.

The growth of 3-4 mm. tips in one week in the standard medium with 4 per cent sucrose has varied between 60 and 95 mm. in the many series which have since been made. The growth of similar roots, under the same conditions but attached to the seed, is about 75 mm. in the first week. The excised roots may therefore be considered as normal in so far as the growth rate is concerned.

**INFLUENCE OF SPECIAL FACTORS.**—Throughout the course of the present work attention was directed toward the various accessory substances or special substances which one might suppose the seedling root normally to receive from the cotyledons. It will be shown

TABLE 8  
EFFECT OF SUCROSE CONCENTRATION UPON GROWTH  
OF EXCISED PEA ROOTS

Percentage sucrose concentration.....	0	0.5	1.0	2.0	3.0	4.0	5.0	6.0
Growth in mm. in one week.....	7	20	29	33	43	56	57	55

in section II that the influence of such factors can be much better studied in roots which have been in culture for some time and in which the supply present in the freshly excised tip has been depleted. Nevertheless for comparison with the work of others it will be of interest to present a few of the results obtained with freshly excised roots. The experiments unless otherwise noted were run with the standard medium and 2 per cent carbohydrate.

The first and obvious experiment was to place pea cotyledons (with the embryo removed) face downward in the petri dish containing roots. Substances necessary for the growth of roots could then diffuse out of the cotyledon and into the medium, whence they might be taken up by the growing root. As shown in table 9, however, living cotyledons give up substances which are inhibitory to the growth of roots, and dead cotyledons give up even more. Recourse was therefore had to peptone and to yeast extract.<sup>1</sup> Peptone definitely

<sup>1</sup> Yeast extract was made by boiling 2 gm. of Fleischman's dry brewers' yeast for 20 minutes, filtering, making up to volume, and diluting with medium to the desired concentration. The concentration of yeast extract is expressed in terms of the original dry weight of yeast. Thus 0.01 yeast extract is the extract of 0.01 gm. of dry yeast in 100 cc. of medium.

increased the growth increment (table 10). The roots receiving 0.08 per cent peptone grew in this case 50 per cent better than the controls. Yeast extract also had a small beneficial effect (table 10), amounting to about 20 per cent for a concentration of 0.02 per cent. Although the improvement in growth resulting from the addition of peptone is marked, and even that resulting from the addition of

TABLE 9  
INFLUENCE OF COTYLEDONS UPON GROWTH OF  
EXCISED PEA ROOTS

	CONTROL WITHOUT ADDITION	FRESH COTYLEDONS ADDED	AUTOCLAVED COTYLEDONS ADDED
Growth in mm. in one week. . . . .	37	28	21

TABLE 10  
INFLUENCE OF PEPTONE AND YEAST EXTRACT UPON GROWTH  
OF EXCISED PEA ROOTS IN 2 PER CENT SUGAR MEDIUM

Percentage peptone con- centration. . . . .	0	0.001	0.005	0.01	0.02	0.04	0.08
Growth in mm. in one week. . . . .	28	29	33	34	35	36	42
Percentage yeast extract concentration. . . . .	0				0.02	0.04	0.08
Growth in mm. in one week. . . . .	30				37	35	33

yeast extract is considerable, neither is spectacular. What effect is present disappears upon still further improvement of the medium, as will be shown later. In part II it will be shown, however, that under the correct conditions both of these crude preparations do possess growth promoting activity.

It was thought that asparagin might prove to be a better source of nitrogen alone. Table 11 shows that it is little if any better. This result might perhaps have been expected since, as has been demonstrated by McKIE and BARNETT (16), the pea seedling is a typical "amino acid" rather than an "asparagin" plant; that is, in the nitrogen metabolism of the pea seedling amino acids rather than asparagin are the chief translocation forms of nitrogen.

Inositol, the well known bios I of EASTCOTT (5), is also without effect upon the growth of pea roots (table 11). Cysteine is markedly inhibitory in concentrations of 10 mg. per liter or more, but seems to have some beneficial effect in lower concentrations. The role of cysteine has not been investigated further.

Root tips grown in 4 per cent sucrose medium do not respond to added yeast extract as do those grown in 2 per cent sucrose medium, as may be seen in table 12. The effect found in the low carbohydrate

TABLE 11  
INFLUENCE OF ASPARAGIN AND OF INOSITOL UPON  
GROWTH OF EXCISED PEA ROOTS

Asparagin concentration . . .	0	1-5 mg./liter	15-50 mg./liter
Growth in mm. in one week	30	33	32
Inositol concentration . . .	0	1 mg./liter	10 mg./liter
Growth in mm. in one week	31	33	31

TABLE 12  
EFFECT OF YEAST EXTRACT UPON GROWTH OF EXCISED  
PEA ROOTS IN 4 PER CENT SUCROSE MEDIUM

Percentage yeast extract concentra- tion . . . . .	0	0.005	0.02	0.08
Growth in mm. in one week . . . . .	75.5	73.5	70.5	64.5

medium may therefore be of a purely nutrient nature. This view is supported by the fact that the highest yeast concentration, 0.08 per cent, is markedly inhibitory in the 4 per cent sucrose medium whereas it is distinctly beneficial in the medium containing only 2 per cent sucrose. It will be shown in section II that yeast extract does contain substances necessary for the continued growth of pea roots. The initial fragment, however, seems to contain sufficient of these substances for a considerable growth if other circumstances are favorable. Essentially the same conclusion has been arrived at by ROBINS (18) for corn roots.

## II. SUBCULTURE OF EXCISED PEA ROOTS

In part I roots grown from freshly excised tips were considered exclusively. Attention will now be turned to the subculturing of

these roots. Transfers (or subcultures) were carried out in essentially the same manner as that described in part I. The same medium containing 4 per cent sucrose was used in 10 cm. petri dishes, twenty to forty roots being used for each part of each experiment. Cultures were again kept in a darkened cabinet and were subject to the same temperature fluctuations. Transfers, unless otherwise specified, were made at approximately weekly intervals.

**INFLUENCE OF YEAST EXTRACT.**—Tips 1 cm. long were cut from roots grown from freshly excised fragments and were transferred again to the standard medium. At the end of the second week this was repeated, and again after the third week. Table 13 gives the

TABLE 13  
SUBCULTURE OF EXCISED PEA ROOTS IN BASIC MEDIUM  
WITHOUT ADDITION OF ACCESSORY FACTORS

EXPERIMENT NO.	GROWTH IN MM. PER PASSAGE			
	1	2	3	4
1. . . . .	65	11	3	0
2. . . . .	63	11	4	0
3. . . . .	75	11	0	0
4. . . . .	59	10	0	0

results of four typical experiments. Passage 1 represents the freshly excised roots; passage 2, the second week; and so on. The growth rate drops very rapidly and becomes practically nil in the third passage. One would first suppose, as ROBBINS (18) did originally, that a substance (or substances) necessary for growth is present in the initial tip in quantities sufficient for the first passage but not for the later ones. This is undoubtedly true in part, but the situation is still somewhat more complicated, as is shown by the following experiment. A large number of freshly excised tips were cultured for one week (passage 1). At the end of this time, when the roots had grown 59 mm., one half of them were subcultured into fresh medium and one half were allowed to remain undisturbed. At the end of the second week the growth increment in passage 1 plus that in passage 2, or the total growth of the transferred roots, was 69 mm.; that of the undisturbed roots was 201 mm. There must then have been



sufficient growth factor present in the initial fragment to permit much more growth than was obtained in the transferred roots. ROBBINS (18) has suggested that the growth factors may be fractionated in some unspecified manner by the process of subculture. Another possibility is that a portion of the active substances is destroyed by the enzymes set free at each new cut surface. Which of these or other possible explanations is the correct one, is as yet unknown.

It has already been pointed out that in the 4 per cent sucrose medium yeast extract has no beneficial effect upon the growth of the initial passage. In marked contrast to this is the effect of yeast extract on the later passages. Table 14 shows the result of an experi-

TABLE 14  
SUBCULTURE OF EXCISED PEA ROOTS IN MEDIUM  
CONTAINING 0.02 PER CENT YEAST EXTRACT

	GROWTH IN MM. PER PASSAGE							
	1	2	3	4	5	6	7	8
No yeast extract.....	59	10	0	0	0	0	0	0
0.02% yeast extract transferred weekly.....	58	33	36	42	54	47	44	57
	(1 & 2)		(3 & 4)		(5 & 6)		(7 & 8)	
0.02% yeast extract transferred each two weeks..	155		112		140		265	

ment in which one series was transferred weekly in medium containing no yeast extract, one series weekly in medium containing 0.02 per cent yeast, and one series every two weeks in yeast medium of the same concentration. As usual, the roots cultured in ordinary medium finished their growth in the third passage. Those in yeast extract on the other hand grew 33 mm. in the second passage but recovered to an average of about 50 mm. per passage for the last six passages. Those roots which were transferred only once in two weeks showed more irregular results but continued to grow excellently.

The total growth increment of the two week roots is again considerably larger than that of the one week roots.

Extensive experiments with different concentrations of yeast extract showed that for long continued subculture, 0.01 per cent yeast

extract is considerably superior to either 0.02 or 0.005 per cent. Roots were therefore cultivated in large numbers in the standard medium with the addition of 0.01 per cent yeast extract. Figure 1 shows the growth per passage of a series which was kept in culture for four months. At the end of this period the roots were perfectly normal and healthy in all respects and their growth rate was quite as high as at the beginning of the period. There seems every reason to

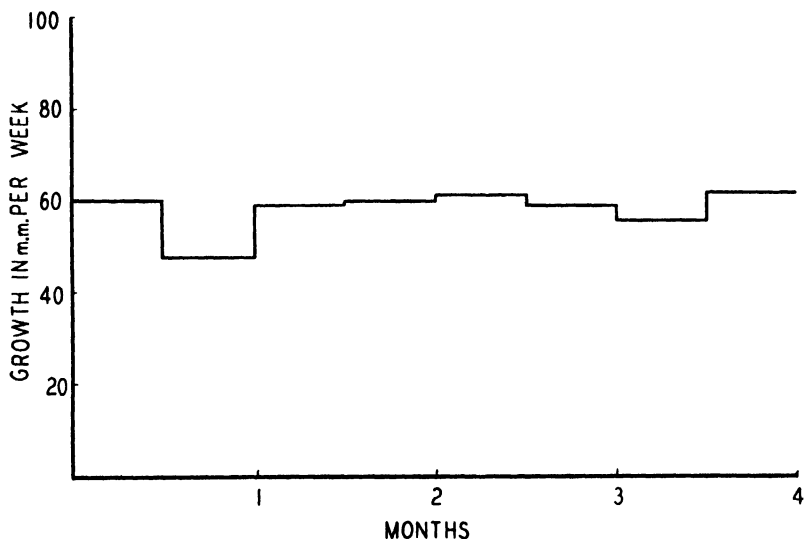


FIG. 1.—Growth rate of excised pea roots in vitro. Cultures transferred weekly in 4 per cent sucrose medium containing 0.01 per cent yeast extract. Means of successive pairs of passages.

suppose that these roots might have been kept in culture indefinitely, but it was necessary to discontinue the series for other reasons. Heretofore WHITE, and recently ROBBINS and BARTLEY (23), have succeeded in keeping tomato roots through several passages without decrease of growth rate. ROBBINS and coworkers (18, 22) have not been able to subculture *Zea* roots indefinitely, and FIEDLER (6) has concluded that for *Zea* roots this is impossible. It seems highly doubtful whether FIEDLER's conclusion is justified. The less satisfactory media used in the early portions of this work (see part I) were incapable of supporting continued growth of pea roots. Only

when a satisfactory medium was found was continued growth possible. It seems likely therefore that the most suitable medium for the cultivation of roots such as those of *Zea* has not yet been found.

ACTIVE PRINCIPLES OF YEAST EXTRACT: VITAMIN B<sub>1</sub>.—A systematic search for the active principles of yeast extract; that is, the substances in yeast extract which are necessary for root growth, was next attempted. At the time the investigation was begun there was no indication as to the possible nature of these substances. During the course of the work, however, several papers bearing upon the subject appeared (21, 23, 22, 31, 1). These will be discussed in connection with the work reported here.

Preliminary experiments indicated that a concentrate of vitamins B<sub>1</sub> and B<sub>2</sub> (Squibb) is considerably more effective than is yeast extract in promoting growth of the later passages of pea roots. Crystalline vitamin B<sub>1</sub>, both the natural and the synthetic product (Merck),<sup>2</sup> was therefore investigated as to its possible accessory growth factor activity. No difference between the natural and the synthetic product was found, and both were highly active, as shown by the following experiment. Freshly excised root tips were cultivated in the standard medium for one week. Since no accessory factors were added, the root's own supply should be depleted and this should allow of a greater response in passage 2. The medium for the second passage consisted of the basic medium plus vitamin B<sub>1</sub> in concentration varying from 20 gamma per cc.<sup>3</sup> to 0.00002 gamma per cc. Controls with 0.01 per cent yeast extract and with no addition were run at the same time. Twenty roots were grown in each set. Table 15 gives the result of a typical experiment. The controls with no addition grew only a small amount as usual, 11 mm. Those with B<sub>1</sub> concentrations greater than 0.0002 gamma per cc. grew even better than the yeast extract controls. A maximum response was attained with 0.0002 to 0.002 gamma per cc., but even 0.00002 gamma per cc. sufficed to elicit a considerable growth response. It is not surprising that pure vitamin B<sub>1</sub> should be able to bring about more growth than is the optimum concentration of yeast extract, since as

<sup>2</sup> We are greatly indebted to the Merck Company for its cooperation in supplying vitamin B<sub>1</sub>.

<sup>3</sup> One gamma is 10<sup>-6</sup> gm.

has already been shown, yeast contains not only beneficial substances but detrimental ones also.

The minimum concentration of vitamin  $B_1$  which is effective in promoting the growth of these roots is about 1-2 in  $10^{11}$ . This activity is of the same order as that found by KÖGL and TÖNNIS (10) for the effect of "biotin" in promoting the growth of yeast. Maximum effectiveness is attained with a concentration of about 1 in  $10^9$ , but no toxic effects have been observed with a concentration of many times this. In later experiments a concentration of 1-2 in  $10^7$  has

TABLE 15  
EFFECT OF CRYSTALLINE VITAMIN  $B_1$  UPON GROWTH OF  
EXCISED PEA ROOTS IN SECOND PASSAGE

$B_1$ concentration yeast/cc. . .	0	0 00002	0 0002	0.002	0 02	0.2	2.0	20 0	0 01% yeast extract
Growth in mm. in one week. . .	11	40	64	70	70	62	68	61	48

regularly been used, since it was desired to leave an adequate margin to allow for destruction, etc.

Crystalline vitamin  $B_2$  (lactoflavin)<sup>4</sup> was also tested for its activity in promoting the growth of pea roots. The sample used did possess some activity, although several thousand times less than that of  $B_1$ . Roots grown with  $B_2$  also never attained the luxuriance of the  $B_1$  roots. It may be that activity of the sample used (a natural product) was merely the result of minute amounts of  $B_1$  present as an impurity. This hypothesis is supported by the fact that added  $B_2$  did not augment the effect of  $B_1$ .

Simultaneously with and independent of the work presented here (and in a preliminary form in an earlier paper, 1), it was found by ROBBINS and BARTLEY (23) that vitamin  $B_1$  is an essential factor for the continued growth of excised tomato roots. The minimum concentration which they found to be effective is of the same order as that found for pea roots. In an earlier paper, however, ROBBINS

<sup>4</sup> The lactoflavin preparation was kindly supplied by Professor LEPKOVSKY, University of California.

and WHITE (22) found that vitamin B (presumably  $B_1$ ) is without effect on the growth of freshly excised *Zea* roots. While a lack of effect upon the freshly excised root would not be surprising, work in this laboratory (unpublished) has indicated that even roots attached to the seed may benefit from additions of  $B_1$ .

It is perhaps well to point out that there is a physiological function for the vitamin  $B_1$  which is normally stored in seeds (24), a

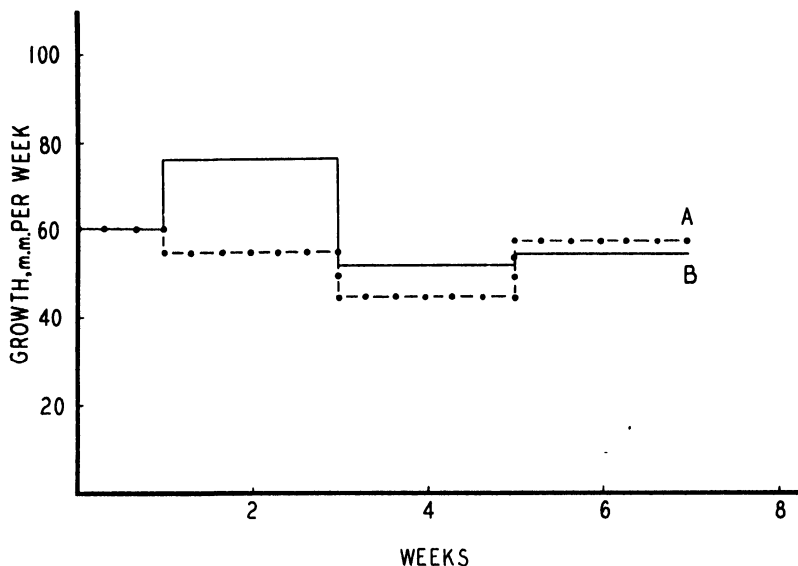


FIG. 2.—Growth rate of excised pea roots in vitro. Cultures transferred weekly in A: medium containing 0.01 per cent yeast extract; B: medium containing 1 in  $10^7$  parts of vitamin  $B_1$ , as accessory growth factors. Means of successive pairs of passages.

conclusion which has been independently arrived at in a different manner by KÖGL and HAAGEN-SMIT (11).

**ACTIVE PRINCIPLES OF YEAST EXTRACT: AMINO ACIDS.**—Pea roots may be carried through several passages in basic medium containing vitamin  $B_1$  as the only accessory factor. This has been indicated in a previous publication (1) and a further example is given in figure 2. During the first four passages the  $B_1$  roots grew better than the controls with yeast extract. After this time, however, growth of the  $B_1$  cultures lagged behind that of the controls and the roots became thin and abnormal in appearance. Such roots nevertheless may be

cultivated for a very long time (fig. 3). This series was transferred weekly for three and one half months. In this case the growth rate became stabilized at about 40 mm. per transfer in the later passages. If this decrease in growth rate during continued subculture is actually due to a second substance or substances, it should be possible to bring about the drop sooner by the use of shorter tips for each transfer. The "dilution" of the second factor should then take place

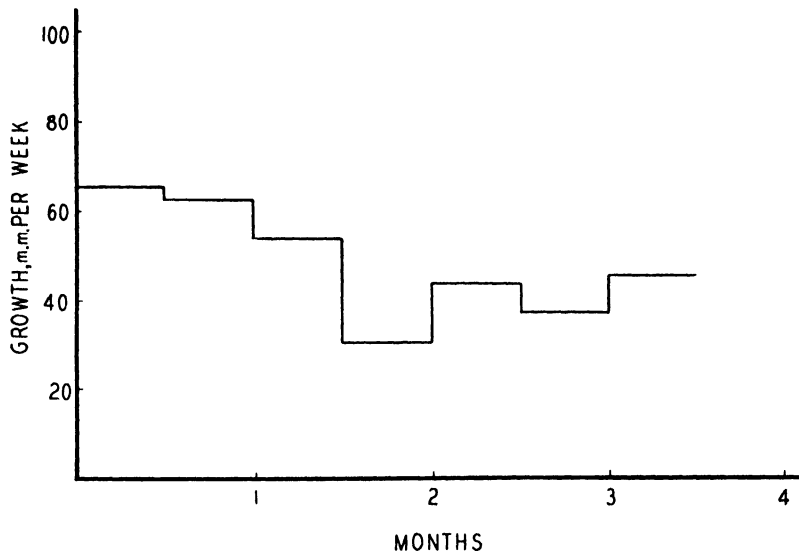


FIG. 3.—Growth rate of excised pea roots in vitro. Cultures transferred weekly in medium containing  $1$  in  $10^7$  parts of vitamin  $B_1$  as accessory growth factor. Means of successive pairs of passages.

more rapidly. Table 16 shows that if 2 mm. tips are used instead of the usual 1 cm. ones, limitation of growth by the second factor does indeed become apparent in the third passage in  $B_1$  medium. On the other hand, 2 mm. tips transferred each time to 0.01 per cent yeast extract medium grow normally although less rapidly than the 1 cm. tips. The individual variation among roots grown from 2 mm. tips, however, was so great as to make their use for further work impracticable. Five to 6 mm. tips were therefore employed for series in which it was desired to make the second factor limiting as soon as possible. With such tips this limitation becomes apparent in general in the fourth passage.

It was first thought that the second factor might be in the nature of inorganic material, for example metallic ions, present in the yeast extracts in small amounts. Yeast ash, however, did not improve in the slightest the growth of the later passages in B<sub>1</sub> medium. ROBINS and coworkers (21) have shown that qualitative filter paper may act as a source of some apparently inorganic growth factor for *Zea* roots. Qualitative filter paper was also without effect as the second factor for pea roots. Other workers (6, 8) have found that indoleacetic acid in small concentrations may greatly increase the

TABLE 16  
GROWTH OF SUBCULTURES OF EXCISED PEA ROOTS;  
TRANSFERS MADE WITH 2 MM. TIPS

	GROWTH IN MM. PER PASSAGE		
	2	3	4
Medium with B <sub>1</sub> . . . . .	43	14	8
Medium with yeast extract . .	38	27	38

growth of excised roots. It is true that their experiments have been done only with the initial passage, and in both cases with *Zea* roots. Indoleacetic acid has been used in the present cultures in concentrations of  $10^{-10}$ ,  $10^{-11}$ , and  $10^{-12}$  molal. These concentrations are of the order of those found to increase the growth of *Zea* roots. In the case of pea roots, however, no effect was found in passages 2 to 12.

It seemed possible that the second factor might merely be nitrogen in the amino form—that these roots might be unable to utilize indefinitely inorganic nitrogen alone, particularly since WHITE (31) has emphasized the importance of amino acids in root growth. Neither alanine nor natural leucine, however, possesses the slightest effect. Asparagin also was without beneficial influence over a wide range of concentrations. In marked contrast to the ineffectiveness of these single amino acids, Witte peptone was found to be quite effective as a supplement to vitamin B<sub>1</sub>. This effect was obtained only over a narrow range of concentrations: 0.01 mg. peptone per cc. was almost without effect, 0.02 and 0.04 mg. per cc. were beneficial, and 0.1 mg. per cc. was markedly toxic. Even in the optimum peptone

concentration the roots in later passages appeared somewhat thicker than normal and were obviously unhealthy. The fact that peptone was active as a B<sub>1</sub> supplement, however, suggested that several amino acids might be needed; that the roots needed not amino nitrogen in general, but rather "essential" amino acids which they are unable to synthesize for themselves. A mixture of amino acids was therefore prepared. Each acid was crystalline and of as high quality as was obtainable. The following list indicates the acids used and their sources. We are greatly indebted to Professor H. BORSOOK for the supply of these amino acids.

glutamic acid hydrochloride . . . . .	c. p. Pfanstiehl
d-l-alanine . . . . .	Fox (made by S. Fox of these laboratories)
l-tyrosine . . . . .	Fox (made by S. Fox of these laboratories)
d-arginine . . . . .	Fox (made by S. Fox of these laboratories)
l-cysteine hydrochloride . . . . .	Fox (made by S. Fox of these laboratories)
glycine . . . . .	Fox (made by S. Fox of these laboratories)
cystine hydrochloride . . . . .	Fox (made by S. Fox of these laboratories)
natural leucine . . . . .	Fox (made by S. Fox of these laboratories)
d-l-serine . . . . .	Amino acid made at the University of California at Los Angeles
d-l-methionine . . . . .	Hoffman LaRoche
d-valine . . . . .	Hoffman LaRoche
d-ornithine dihydrochloride . . . . .	Hoffman LaRoche
d-lysine dihydrochloride . . . . .	Hoffman LaRoche
l-histidine monohydrochloride . . . . .	Hoffman LaRoche
tryptophane . . . . .	c. p. Pfanstiehl
asparagine . . . . .	Merck

Equal amounts of each amino acid were weighed out, dissolved together, and various amounts of the mixture used either alone in the basic medium or as a supplement to vitamin B<sub>1</sub>. With this mixture, as with peptone, the range of concentrations which could be used was narrow. It was found that 0.02 mg. per cc. was somewhat toxic;



0.002 mg. per cc. had little effect; but 0.01 mg. per cc. gave very satisfactory results (table 17). The vitamin B<sub>1</sub> series of this experiment grew, as usual, very well during the first three passages. In the fourth, fifth, and sixth passages it dropped to the lower growth rate characteristic of roots having B<sub>1</sub> as the only accessory factor.

The amino acid mixture alone was also beneficial but the roots became thin and were in poor condition at the end of the sixth passage. The combined effect of vitamin B<sub>1</sub> and the amino acid mixture was striking. These roots elongated rapidly during the entire six passages. They furthermore formed secondaries which the slower

TABLE 17  
SUBCULTURE OF EXCISED PEA ROOTS; EFFECTS OF  
VITAMIN B<sub>1</sub> AND AN AMINO ACID MIXTURE,  
SINGLY AND TOGETHER

	GROWTH IN MM. PER PASSAGE				
	2	3	4	5	6
B <sub>1</sub> alone . . . . .	58	68	35	34	36
Amino acid mixture alone	66	78	50	51	32
B <sub>1</sub> and amino acid mixture together . . . . .	68	77	77	67	83

growing control series did not; they were of normal diameter and appeared to be in every way in the best of condition. It was unfortunately necessary for other reasons to interrupt this experiment after the sixth passage (and to terminate another series which had been through eight passages). Further experiments must therefore show whether pea roots may be subcultured for longer periods in this medium of completely known composition. There is at present every indication that they can be subcultured indefinitely. Roots grown in this medium are equal or superior to those grown in yeast extract, and there has been no sign of decreased growth rate in the later passages. Further experiments must also determine which of these amino acids are the essential ones, and the optimum concentration of each must be found.

That a complex mixture of amino acids, in addition to vitamin B<sub>1</sub>, should be necessary for the continued growth of excised pea roots is not surprising. Amino acids are formed in large amounts in

the germinating pea (16) and they might be expected to possess some value for the seedling plant. The root of the mature plant also is "heterotrophic," in that it depends upon the green aerial portions for its supply of carbohydrates. One can easily imagine that there are also particular amino acids which the root is unable to synthesize and which must be supplied by the leaves. WHITE (31) has recently found that a complex mixture of amino acids is necessary for the growth in vitro of excised tomato roots. In his case also the amino acids act in conjunction with another factor which has not yet been identified but which from the work of ROBBINS and BARTLEY (23) and of BONNER (1) appears to be vitamin B<sub>1</sub>.

### Discussion

This investigation has shown that if a suitable medium is used, excised pea roots grow rapidly in length in vitro. Tips freshly excised from the plant grew, under the conditions used, 60 to 95 mm. in the first week, and formed one to four short lateral roots. Under less favorable conditions, for example, in the 2 per cent glucose medium rather than in the 4 per cent sucrose medium, growth in length was less and no secondaries were formed.

This lack of lateral formation in unsuitable media no doubt accounts for the fact that KOTTE (13), who obtained only 10–14 mm. growth in twelve days, never observed lateral formation on excised pea roots. If freshly excised tips were allowed to remain in the best medium for two (rather than one) weeks, the growth in length was 200 mm. or more, and as many as twenty-five laterals were formed on a single root. This secondary root formation also took place in the later passages. Roots giving good growth rates (in suitable medium containing 0.01 per cent yeast extract) formed one to four laterals in one week. The roots in the series of table 14 which were transferred only once in two weeks formed as many as twenty-five laterals per root in the fourth passage. Many attempts have been made to subculture these lateral growing points but without consistent success. An occasional lateral tip developed into a normal root, but the majority grew either slowly or not at all. Those which grew also remained thinner than normal.

This is an indication that even the best yeast extract medium is not optimal for the growth of excised pea roots, but further experi-

ments must determine what improvements can be made. Since it has been impossible to subculture the lateral growing points it has been impossible as yet to establish clones of pea roots, as has WHITE (29) with tomato roots.

It has long been known that crude extracts, and in particular yeast extract, are beneficial and even necessary for the growth of excised roots. Pea roots are no exception to this general rule. Of greater interest is the fact that it has been possible to substitute known chemical substances for the unknown "active principles" of yeast. Crystalline vitamin B<sub>1</sub> has been shown to be an accessory factor, a special chemical substance necessary, in minute amounts, for the growth of pea roots. As a complement to vitamin B<sub>1</sub>, and necessary for continued growth, specific amino acids are also required in small amounts. Apparently it might equally well be said that vitamin B<sub>1</sub> acts as a complement to the specific amino acids. In any case, either alone is inadequate and only their combined activity will support the continued growth of pea roots. It has been said, concerning the cultivation of roots in vitro, that "The proved unlimited capacity for growth of such cultures obviously sets aside the concept of indispensable and *specific* correlation hormones" (30). As the writers view the matter, exactly the reverse is more obvious. Whether we call vitamin B<sub>1</sub> and the specific amino acids phyto-hormones or accessory growth substances, they are indispensable to the continued growth of these root cultures, and they must be, in normal plants, carriers of growth correlation between root and seed or shoot.

### Summary

1. A medium suitable for the growth of excised pea roots has been described. This medium contains 4 per cent sucrose in addition to inorganic salts.
2. Freshly excised pea root tips 3-4 mm. long grow 60 to 95 mm. in this medium in the first week; 200 mm. or more in the first two weeks.
3. Yeast extract (0.01 per cent) must be added to the basic medium if good growth in passages after the first is to be obtained. Roots have been kept in culture with weekly transfers for four months on the basic medium with added yeast extract.
4. Crystalline vitamin B<sub>1</sub> is capable of partially replacing yeast

extract; vitamin B<sub>1</sub> alone is not able to support the continued optimal growth of excised pea roots in later passages.

5. A mixture of pure, crystalline amino acids is capable of replacing that portion of the activity of yeast extract which is not due to vitamin B<sub>1</sub>. With the use of vitamin B<sub>1</sub> and this mixture of amino acids a highly satisfactory, completely known medium for the cultivation of excised pea roots has been obtained. This medium of known composition supports the growth of such roots as well or better than media containing yeast extract.

6. Substances such as vitamin B<sub>1</sub>, which are necessary in minute amounts for the growth of the isolated root but which are normally supplied by some other portion of the intact plant, are carriers of a growth correlation and hence are to be regarded as phytohormones.

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# EFFECT OF INDOLEACETIC ACID ON GROWTH AND CHEMICAL COMPOSITION OF ETIOLATED BEAN PLANTS<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 483

JOHN W. MITCHELL AND WILLIAM E. MARTIN

(WITH FOUR FIGURES)

## Introduction

In connection with recent investigations which show that hormones have a marked effect upon the morphological and histological development of certain plants (2), it was considered of interest to study the effect of 3 per cent  $\beta$ -indoleacetic acid on the chemical composition of plants treated with it. To facilitate such a study several thousand bean plants were grown in the dark. The synthesis of organic foods in addition to those already present, which might complicate the study, was eliminated in this way. Some of the plants were treated with indoleacetic acid and others were used as controls. The wet weight, dry weight, volume, sugar, starch, and nitrogen content of various portions of the treated and untreated plants were compared at the beginning and end of a four day period following treatment.

## Investigation

**MATERIALS AND TREATMENT.**—Kidney beans, *Phaseolus vulgaris*, were used in the experiments. A great number of seeds were carefully selected for uniformity and then weighed individually, rejecting all that varied more than 50 mg. from the mean. In each experiment 1050 seeds were planted at a uniform depth in unsterilized quartz sand contained in fifty 12 inch clay pots. They were watered with warm tap water and placed in the dark at 80° F. with a relative humidity of 70–80 per cent saturation.

The plants were usually above the sand level on the third day

<sup>1</sup> This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

after planting, and on the fourth day the seed coats were removed. The cotyledons and epicotyls were thus freed so that further growth was not impeded by the mechanical obstruction of the seed coats. By the following day the plants were usually 6–7 inches high and the first internode from 0.5 to 1 inch in length.

Seven to eight hundred uniform plants were then selected for treatment. One third of these were treated by spreading approximately 0.05 cc. of 3 per cent  $\beta$ -indoleacetic acid lanolin mixture on the two opposite sides of the first internode. In this way about one third of a square centimeter of epidermis was covered with paste. The remaining two thirds of the plants were treated in a similar way by placing an equal amount of pure lanolin on the first internode. One half of the latter group was harvested immediately as initial controls, while the remaining plants, composed of an equal number of controls and treated, were allowed to remain in the dark under controlled conditions for four days, and then harvested.

**PHYSICAL MEASUREMENTS.**—At harvest the plants were first washed free from most of the sand, then dipped into a saturated solution of sodium chloride to loosen the remaining sand adhering to the roots. The plants were washed carefully and divided into the following fractions: (1) roots, (2) hypocotyls, (3) cotyledons, (4) first internodes, (5) second internodes together with the petioles and leaves.

The fresh weight of each fraction, representing the parts of approximately 250 plants, was then recorded and the volume measured by the displacement of water. In this way both the fresh weight and the volume were determined for each of the five fractions.

Immediately following these determinations the fractions were chopped finely and placed in a well ventilated drying oven at 80° C. for 20 hours. The dry weight of each fraction was then determined by weighing the samples in large weighing bottles on an analytical balance.

In all experiments the results were obtained from samples representing at least 225 plants. Preliminary experiments showed that 225 plants were a sufficient number to give accurate results. As a matter of convenience, some of the data are presented on the basis of 100 plants.

**CHEMICAL METHODS.**—Prior to analysis, all samples were ground by means of a coffee mill and then in a ball mill until the material would pass through an 80 mesh sieve. The ground material was then redried at 80° C. before samples were taken for analysis. The soluble sugars were removed from weighed samples of the dry powder by means of repeated extractions with 80 per cent alcohol, seven such extractions sufficing to remove the sugars and obtain a negative alpha naphthol carbohydrate test.

The extract so obtained was evaporated on a steam bath, and the alcohol replaced with distilled water. This aqueous extract was then cleared and the sucrose hydrolyzed by invertase, according to the procedure outlined by LOOMIS and SHULL (3). Following inversion, the solutions were made to volume and sugars determined according to the method of PHILLIPS (5).

The starch remaining in the residue after alcoholic extraction was hydrolyzed by fresh saliva as outlined by LOOMIS and SHULL, and the sugar in the final solution determined by the method of PHILLIPS. Starch values so obtained were expressed as glucose.

The total nitrogen content of the dry powders from the several regions of the plants was determined by the Gunning method, modified to include nitrates as described in the Official Methods (1). At the end of the experiment in both treated and untreated plants considerable quantities of nitrate nitrogen were found, especially in the hypocotyls. The amount present was considerably greater than the quantity which could have been supplied through the use of tap water.

### Results and discussion

**MORPHOLOGICAL OBSERVATIONS.**—The first apparent reaction of plants treated with indoleacetic acid occurred within 24 hours after treatment, and was evidenced by a marked bending of the hypocotyls at a point about 2-4 inches below the cotyledons. This reaction was only temporary, and within 96 hours the hypocotyls of treated plants were nearly straight. Noticeable swellings of the first internodes occurred at the points where indoleacetic acid was applied, and in many cases the hypocotyls became enlarged immediately below the cotyledons within 36 hours after treatment. During the four days following treatment the first internodes (the point of treat-



ment) elongated very little, while those of control plants increased from an initial length of 1 inch up to 5 inches at the end of the experiment. The second internodes, petioles, and leaves of treated plants did not increase appreciably in size, while there was a marked increase in the size of these portions of control plants during the same period. There was little difference in the apparent size of the roots or hypocotyls of treated as compared with control plants, even at the end of the experiments. In many cases dense rows of roots were developed along the entire length of the hypocotyls. This inhibitive effect upon the elongation of plants grown in continuous darkness is in contrast to the effect observed in the case of plants similarly treated but grown under the conditions of alternating night and day that obtained in late May and early June in the greenhouse at Chicago (figs. 1, 2).

The histological responses and details shown by the treated regions closely resemble those shown by plants of comparable age grown under conditions of alternating light and darkness. The differences between treated and untreated plants grown continuously in darkness, however, are very great (figs. 3, 4).

**WEIGHTS, VOLUMES, AND CHEMICAL COMPOSITION.**—In general the analyses showed (1) that in the case of both treated and control plants, materials were rapidly moved from the cotyledons into other parts of the plants during the four days following treatment; (2) that a greater quantity of material was withdrawn from the cotyledons of control plants than was withdrawn from the cotyledons of treated plants during the same period; and (3) that indoleacetic acid as used in these experiments determined to a large extent the direction of flow of materials from the cotyledons.

Thus there was an appreciable decrease in the fresh and dry weight of the cotyledons of both control and treated plants, with an accompanying gain in the fresh and dry weight in at least some of the other parts of the plants (table 1). Starch, sugars, and nitrogenous compounds were transferred from the cotyledons into the different parts of the newly developed plants (table 2), so that some of these parts gained dry matter.

That a greater quantity of these materials was withdrawn from the cotyledons of control plants than was withdrawn from the

TABLE 1

SUMMARY OF EXPERIMENT 5 SHOWING EFFECT OF INDOLEACETIC ACID ON FRESH AND DRY WEIGHT OF DIFFERENT PARTS OF ETIOLATED BEAN SEEDLINGS; FIGURES EXPRESSED IN TERMS OF GRAMS PER 100 PLANTS

REGION OF PLANT	INITIAL CONTROL		FINAL CONTROL		TREATED	
	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT
Roots.....	81.6	4.94	85.0	3.53	94.4	3.06
Hypocotyls.....	342.0	17.47	394.7	18.06	420.8	22.72
Cotyledons.....	91.2	26.21	42.7	7.43	48.6	9.57
First internodes.....	15.0	1.44	146.8	9.73	99.8	6.72
Second internodes and leaves.....	51.2	6.54	146.7	10.70	50.5	6.33
Total.....	581.0	56.60	815.9	49.48	723.1	49.30

TABLE 2

EFFECT OF INDOLEACETIC ACID ON DISTRIBUTION OF SOME CHEMICAL CONSTITUENTS OF ETIOLATED BEAN SEEDLINGS; VALUES OF DUPLICATE DETERMINATIONS EXPRESSED AS GRAMS PER 100 PLANTS

REGION	TOTAL NITROGEN			SOLUBLE SUGARS			STARCH		
	INITIAL CON-TROL	FINAL CON-TROL	TREAT-ED	INITIAL CON-TROL	FINAL CON-TROL	TREAT-ED	INITIAL CON-TROL	FINAL CON-TROL	TREAT-ED
Roots.....	{ 0.20 0.20	{ 0.15 0.15	{ 0.18 0.18	{ 0.10 0.10	{ 0.03 0.03	{ 0.03 0.03	None	None	None
Hypocotyls.....	{ 1.31 1.31	{ 1.54 1.51	{ 1.85 1.85	{ 1.66 1.63	{ 1.27 1.38	{ 2.38 2.39	None	None	None
Cotyledons.....	{ 1.20 1.20	{ 0.18 0.18	{ 0.35 0.35	{ 1.73 1.71	{ 0.63 0.63	{ 0.84 0.82	7.17 7.51	0.14 0.12	0.74 0.71
First internode....	{ 0.08 0.08	{ 0.57 0.58	{ 0.42 0.43	{ 0.07 0.07	{ 0.76 0.77	{ 0.31 0.32	None	None	None
Second internode and leaves.....	{ 0.49 0.49	{ 0.88 0.88	{ 0.54 0.53	{ 0.04 0.04	{ 0.58 0.59	{ 0.17 0.12	None	None	None
Whole plant..	3.28	3.31	3.34	3.57	3.33	3.70	7.34	0.13	0.73



FIG. 1.—Stems of plants grown under natural light conditions: 2 and 4 treated laterally with a ring of 3 per cent indoleacetic acid lanolin mixture; 1 and 3 untreated. All thirteen days after treatment. Apart from formation of tumors and adventitious roots on treated stems, the two types were identical, with large green leaves and beginnings of flowering branches. From HAMNER and KRAUS (2).

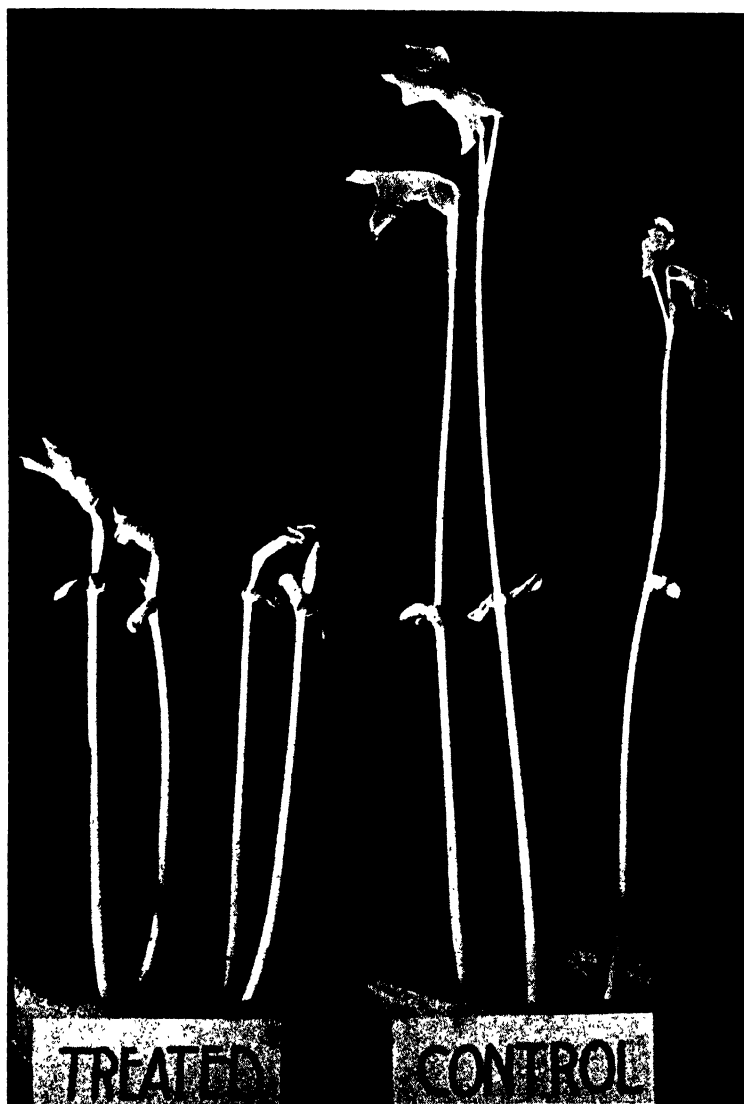


FIG. 2.—Plants grown in continuous darkness four days after lateral application of lanolin mixture to first internode. After treatment there was but slight elongation of the first internode but marked increase in diameter, and many small lateral roots developed in the treated region. Histological details of the two types of stems are shown in figs. 3 and 4.



FIG. 3.—Transverse section through treated portion of plant grown in continuous darkness, four days after treatment. Pith cells living, but not meristematic; cambium and phloem tissues markedly proliferated; xylem not distorted; root differentiated from ray and phloem derivatives, and cells of pericycle and cortex disintegrating.

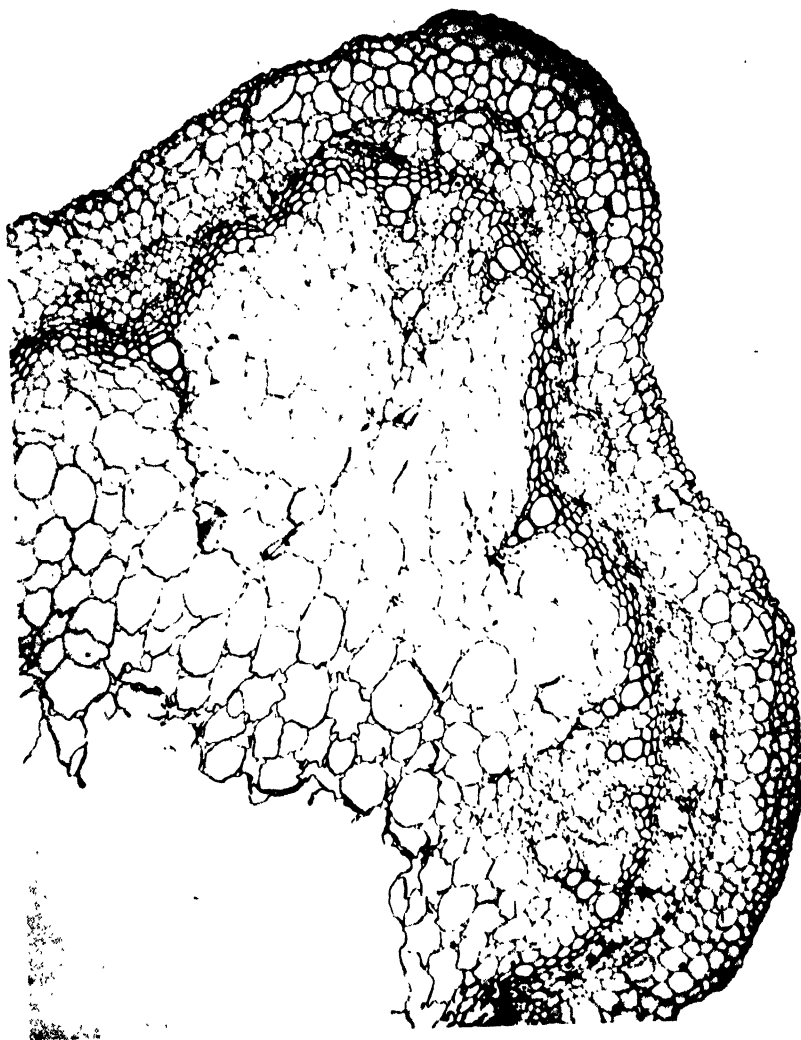


FIG. 4.—Transverse section through comparable portion of control plant grown under same environmental conditions as plants shown in fig. 3. Dead pith cells surrounding a central cavity; few derivatives have differentiated from the cambium, those of the xylem beginning to lignify; phloem parenchyma not proliferated; ray cells not meristematic; pericyclic, cortical, and epidermal cells not disrupted.

cotyledons of treated plants is evidenced, first by the loss of dry weight. In a study of about 2800 plants the results, expressed on the basis of 100 plants, show that the cotyledons of control plants decreased 69.20 gm. in dry weight during four days following treatment, while those of treated plants decreased only 64.26 gm. during the same time (table 3). Volume measurements of the cotyledons showed similar differences (table 4).

Further evidence of the inhibitory effect of indoleacetic acid upon the withdrawal of materials from the cotyledons is apparent from a

TABLE 3

SUMMARY OF EXPERIMENTS ON EFFECT OF INDOLEACETIC ACID ON DRY WEIGHT OF COTYLEDONS AND ENTIRE PLANTS; FIGURES BASED ON APPROXIMATELY 3700 PLANTS AND EXPRESSED AS GRAMS PER 100 PLANTS

EXPERIMENT	COTYLEDONS			ENTIRE PLANT		
	INITIAL CONTROL	FINAL CONTROL	TREATED	INITIAL CONTROL	FINAL CONTROL	TREATED
1.....				37.00	33.20	33.40
2.....	25.52	6.45	7.34	48.16	43.14	44.13
3.....	22.40	6.96	7.73	52.29	47.08	46.24
4.....	22.40	6.46	7.62	48.14	41.94	43.38
5.....	26.21	7.46	9.57	56.60	49.48	49.30
Total....	96.53	27.33	32.27	242.19	214.84	216.45

consideration of the starch, sugar, and total nitrogen content of the cotyledons of treated as compared with those of control plants. On the basis of 100 plants, the cotyledons of the treated contained, at the end of the experiment, approximately 0.73 gm. of starch while those of control plants contained only 0.14 gm., expressed in terms of glucose. Cotyledons of 100 treated plants contained approximately 0.83 gm. of sugar and 0.35 gm. of nitrogen, while those of 100 control plants contained only 0.63 and 0.18 gm. of sugar and nitrogen respectively. The difference in the rate at which these materials were removed from the cotyledons, however, was not associated with a measurable difference in the rates of respiration of treated as compared with control plants, as will be mentioned later.

It is also evident that the application of indoleacetic acid appre-

ciably affected the amount of water absorbed by the plants as a whole. In the four days following treatment, control plants absorbed 62 per cent more water than did treated plants.

It has been pointed out that, following treatment, a large amount of the materials contained within the original seeds was transported from the cotyledons of both treated and control plants into the other parts of the plants. There were marked differences, however, between the places in which these materials were finally deposited in the control as compared with treated plants. Thus in experiment 5

TABLE 4

EFFECT OF INDOLEACETIC ACID ON VOLUME OF DIFFERENT  
PARTS OF ETIOLATED BEAN SEEDLINGS

REGION	INITIAL CONTROL (CC.)	FINAL CONTROL (CC.)	TREATED (CC.)
First internode*	15	167	110
Total above cotyledonary plate		368	235
Cotyledons		47	55
Total below cotyledonary plate		534	557
Entire plant	637	902	782

\* Portion of plant treated.

(table 1) that portion of control plants above the first internode gained approximately 64 per cent in dry weight, due to the deposit of materials transferred from the cotyledons during four days following treatment. In contrast to this, comparable parts of treated plants lost approximately 3 per cent of their original dry weight during the same time (table 1). The first internodes of treated plants gained in dry weight following treatment, but this gain was considerably less than the increase that was apparent in the first internodes of control plants. On the other hand, hypocotyls of treated plants gained more in dry weight following the application of indoleacetic acid than did similar parts of control plants. There was little difference in the dry weight of roots of treated as compared with control plants.

A comparison of the starch, sugar, and nitrogen content of the different parts of the plants leads to the same conclusion, namely, that indoleacetic acid applied to the first internode of etiolated plants inhibited the deposition of materials in the treated portions



and parts of the plants above the point of treatment. Materials which moved in the control plants from the cotyledons principally into the first internodes, second internodes, petioles, and leaves were deposited in the case of treated plants largely in the hypocotyls and first internodes. The volume of that portion of control plants above the cotyledons increased much more than did the volume of the same portion of treated plants following the application of indoleacetic acid (table 4). Transverse sections made through the treated portion of etiolated plants at the end of the experiment showed no apparent evidence of mechanical blockage or inhibition of development of the conductive tissues.

Although indoleacetic acid caused a marked difference in the morphological and histological development and in the rate and direction of transport of carbohydrate and nitrogenous compounds of the plants, it did not greatly affect their rate of respiration. Thus data collected from a study of approximately 3600 plants showed that the controls decreased 5.47 gm. in dry matter per 100 plants, due to respiration during the four days following treatment. The same number of treated plants lost 5.14 gm. during the same period. The difference in these values is not considered significant as it falls within the limits of experimental error.

In preliminary experiments the amount of carbon dioxide respired by treated and untreated etiolated beans was measured by the method described by MITCHELL (4). No significant differences were noted in the amount of carbon dioxide respired by the treated as compared with the untreated plants during a period of seven days.

### Summary

1. Three per cent  $\beta$ -indoleacetic acid lanolin mixture applied to the first internode of etiolated bean seedlings caused the formation of galls at the point of application and the development of roots in the galls, and in many cases dense rows of roots developed the entire length of the hypocotyls.

2. The first internodes, second internodes, petioles, and leaves of treated plants increased less in volume, fresh weight, dry weight, and length than did corresponding parts of control plants.

3. The histological responses shown by treated regions of plants

grown in continuous darkness closely resemble those shown by plants of comparable age grown under alternating light and darkness, as reported by other investigators. Histological differences between treated and untreated plants grown in continuous darkness were very great.

4. The application of indoleacetic acid to the first internode of etiolated plants retarded the transport of materials from the cotyledons and also the uptake of water by the plants.

5. Analyses of treated and untreated plants that included the determination of fresh weight, dry weight, volume, starch, sugar, and nitrogen contained in different portions of the plants showed that indoleacetic acid greatly affected the direction of transport of materials from the cotyledons. Materials were conducted from the cotyledons upward only as far as the first internode (the point of treatment) in the case of treated plants. At the end of the experiments, transverse sections through the treated portion of plants grown in the dark showed no apparent evidence of mechanical blockage or inhibition of development of the conductive tissues.

6. No significant differences were observed in the amount of dry matter respired by treated as compared with control plants.

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# PARTHENOCARPIC FRUITS INDUCED BY SPRAYING WITH GROWTH PROMOTING COMPOUNDS

F. E. GARDNER AND PAUL C. MARTH

(WITH FOUR FIGURES)

## Introduction

Parthenocarpy occurs naturally in a number of plant species and by a variety of means has been induced artificially in others. Recently GUSTAFSON (2) obtained fruit development in several species which normally do not exhibit parthenocarpy by applying lanolin mixtures of growth promoting compounds on the styles, which had first been cut off close to the ovaries. HAGEMANN (3) also obtained parthenocarpic fruits in the case of *Gladiolus* by applying to the stigmas indoleacetic acid in a lanolin paste. The present report is an account of experiments in producing parthenocarpic fruits by means of spraying the blossoms with aqueous solutions of several well known growth promoting compounds. It is concerned chiefly with the American holly, *Ilex opaca*, but includes also results with the strawberry, the apple, and the grape.

The American holly is particularly well adapted for experiments on parthenocarpy in that it is dioecious, and the pistillate plants, which of course require no prior emasculation, can easily be protected from chance pollination. Moreover no development of the fruit, such as is common in unpollinated cucurbits, has previously been observed to occur in *Ilex opaca* without pollination, despite frequent attempts to induce fruit setting artificially. In some species, however, for example *I. cornuta*, parthenocarpy is not uncommon.

Experimental work on the various steps necessary in the production of small, fruit bearing plants of the American holly has been in progress for several years. Such plants, with their attractive green leaves and contrasting red fruits, are of value to the florist during the Christmas season. One of the laborious steps in the production of good plants has been hand pollination in order to insure a full set of

"berries."<sup>1</sup> In the search for an easier and less time consuming method of insuring fruit setting, unsuccessful attempts have been made to bring about parthenocarpy by dusting the open pistillate flowers with irritants, such as sulphur. Miscellaneous pollens, including that from the rose, daisy, iris, and dandelion, have also been applied but no fruits resulted. The application of holly pollen, however, normally resulted in 100 per cent fruit set.

### Investigation

The holly plants used in the experiments on parthenocarpy which follow were propagated in September, 1936, from a single large pistillate tree. When holly trees are of bearing age the entire new shoot structure with its flower primordia is determined in the buds during the previous summer. The flush of growth made in the spring is therefore simply the unfolding and development of structures already preformed and is accomplished in two or three weeks. Ordinarily there is no further shoot elongation during the year, except under certain conditions to be mentioned later in this report.

When well rooted, the holly cuttings were potted and placed in a protected coldframe over winter in order to insure a normal termination of the rest period. In the spring they were moved to the greenhouse, where several inches of new growth was produced, and on this the flowers were borne. When in bloom, the plants were sprayed with dilute aqueous solutions of the following four synthetic compounds: indoleacetic, indolepropionic, indolebutyric, and naphthaleneacetic acids.

No alteration of the floral parts, such as shortening of the style, was deemed necessary in the case of holly, which has a broad stigma and an extremely short style (fig. 1). This characteristic may be an important factor in the parthenocarpic response obtained with the growth promoting compounds.

**SPRAYING OF BLOSSOMS OF DIFFERENT AGES.**—Under greenhouse conditions, the pistillate holly flower remains open about one week, after which time the corolla withers and the pistil and pedicel become yellow before abscission. Before testing the relative effectiveness of various growth substances in causing parthenocarpy, it was desired

<sup>1</sup> Botanically the holly fruit is a compound drupe.

to determine the response of flowers of different ages, since this might be an important consideration in subsequent comparisons. Accordingly some plants were given a single spraying of 0.04 per cent indoleacetic acid in water before the flowers were open; that is, while the four petals of the corolla still completely covered the pistil. Other

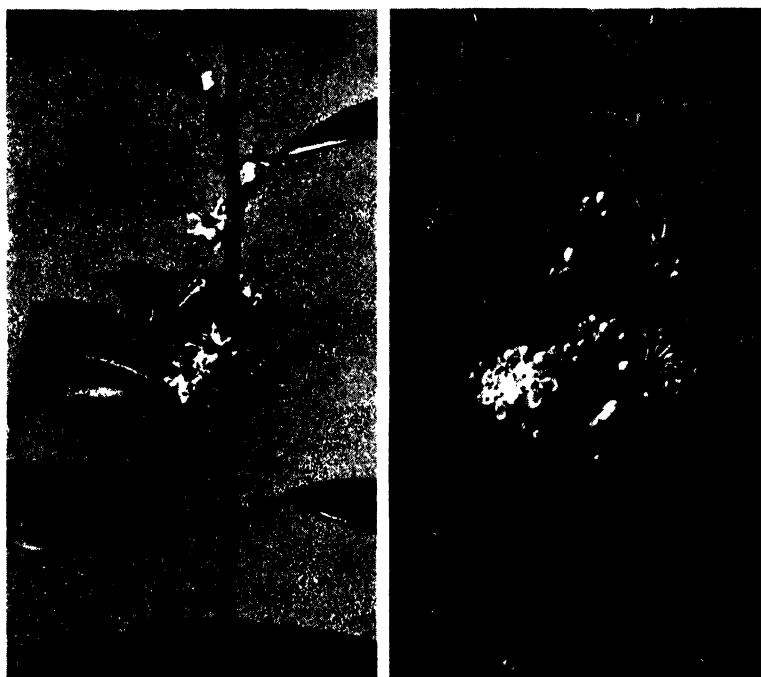


FIG. 1.—Left: pistillate plant of *Ilex opaca* showing flowers borne singly, broad stigmas, short styles, and anthers entirely devoid of pollen. Lowest flowers denote approximate point where current growth begins. Right: staminate plant showing flowers borne in groups (usually of three), absence of pistils, but well developed anthers full of pollen.

plants were sprayed when the flowers had been open for one, three, five, and seven days respectively. All flowers which were open so that the spray could reach the pistil, responded equally well in development of parthenocarpic fruits. Flowers sprayed in the bud stage failed entirely to develop fruit, however, indicating that the stimulus is best transmitted through the pistil rather than through the heavy cuticle of the pedicel or other organs.

Although holly flowers remain in bloom for approximately a week, at any time during which period they are equally responsive to applications of indoleacetic acid, they also responded, at least in the case of naphthaleneacetic acid, several days after the corolla had completely withered and the flowers were ready to fall. Several plants were allowed to reach this stage in flowering and then sprayed with an aqueous solution of 0.006 per cent naphthaleneacetic acid. The pedicels and pistils had become yellow, giving the impression that the stigmas were no longer receptive. Within two days after spraying, however, the pedicels and pistils became green in color and fruits were subsequently developed.

#### COMPARATIVE EFFECTIVENESS OF SEVERAL GROWTH SUBSTANCES.

--For these comparative tests sets of plants were selected having approximately the same number of open flowers. In order to facilitate the recording of number of flowers and fruits set per plant, all unopened flowers were first removed. The percentages of flowers which set fruit, shown in table 1, are based on from thirty to thirty-six flowers about equally distributed on four plants for each concentration shown. The four growth substances indicated were applied only once, care being taken that every flower was thoroughly sprayed.

The work of AVERY, BURKHOLDER, and CREIGHTON (1), together with that of ZIMMERMAN and HITCHCOCK (4), shows that the relative effectiveness of the growth promoting compounds in one plant response may be very different if measured in terms of some other reaction of the plant. To the evidence already available on the relative potency of the growth promoting substances in the various plant responses heretofore studied can now be added information relative to their effectiveness in parthenocarpy.

It is apparent from table 1 that, of the four compounds used, naphthaleneacetic acid was by far the most effective, in terms of concentration, in producing parthenocarpy in holly. Even at a dilution of 1 part per million an appreciable number of flowers set fruit. It is difficult to give the other three compounds a relative rating on the basis of the data presented, inasmuch as 100 per cent fruit set was not obtained with either indoleacetic or indolepropionic acids, yet both compounds caused some fruit setting at appreciably lower con-

centrations than did indolebutyric acid. It is probable that other factors, such as the rapidity of fruit development and the percentage of fruit which later abscised, should also be considered along with the percentage set, in any attempt to evaluate the relative effectiveness of these three compounds in causing parthenocarp. These factors are to be discussed presently.

The three highest concentrations of indolebutyric acid which resulted in 100 per cent fruit set also caused marked epinasty of the

TABLE 1  
PERCENTAGE FRUIT SET IN RELATION TO EACH OF FOUR COM-  
POUNDS IN AQUEOUS SOLUTION OF VARIOUS CONCENTRATIONS

PERCENTAGE CONCENTRATION	PERCENTAGE SET			
	INDOLE- ACETIC	INDOLE- BUTYRIC	INDOLE- PROPIONIC	NAPHTHA- LENEACETIC
0.1.....	84.8	100	41.9	.....
0.08.....	53.6	100	65.6	.....
0.06.....	64.3	100	68.9	.....
0.04.....	54.5	60.6	54.8	.....
0.02.....	38.2	21.2	32.2	.....
0.01.....	9.0	0	22.5	100
0.008.....	3.2	0	0.	100
0.006.....	3.0	0	3.1	100
0.004.....	6.0	0	12.1	97.2
0.002.....	0	0	.....	74.2
0.001.....	.....	0	.....	52.8
0.0008.....	.....	.....	.....	30.0
0.0005.....	.....	.....	.....	16.6
0.0003.....	.....	.....	.....	23.3
0.0001.....	.....	.....	.....	10.7

leaves. This was the only compound, and then only at the higher concentrations, which caused any observable disturbance of the foliage.

INFLUENCE OF REPEATED SPRAYINGS.—In the preceding comparison of the effectiveness of the several compounds, only one application of the spray was made, on the supposition that repeated sprayings might possibly interfere with an evaluation of the various concentrations tried. To determine the effect of repeated stimulus, however, the indoleacetic acid concentration series was applied four times in another group of plants at intervals of 24 hours, to compare

the results with those of a single application. Table 2 shows this comparison, the results with the single spraying being the same data appearing in table 1.

It is evident from table 2 that the repeated stimulus of successive sprayings with low concentrations is much more effective in parthenocarp than single applications. In the practical use of these compounds in producing fruit on holly, successive sprayings would be necessary in that the blossoms are not all open at one time.

DEVELOPMENT OF PARTHENOCARPC FRUITS.—Not only was naphthaleneacetic acid most effective at the lower concentrations in causing a high percentage of fruit setting but also in the rapidity of

TABLE 2  
PERCENTAGE OF FRUIT SET RESULTING FROM SINGLE VERSUS  
REPEATED SPRAYS OF INDOLEACETIC ACID

NO. OF SPRAYINGS	PERCENTAGE CONCENTRATIONS OF INDOLEACETIC ACID					
	0.02	0.01	0.008	0.006	0.004	0.002
1. . . . .	38.2	9.0	3.2	3.0	6.0	0
4. . . . .	79.4	36.3	30.3	12.1	17.6	0

its action in bringing about a greening of the pedicels and ovaries and a prompt swelling of the latter. Within two days after spraying with naphthaleneacetic acid development of the ovaries was easily discernible. This stimulation was much more rapid than that following pollination. The relative rapidity of reaction to the other acids, in descending order, was indolebutyric, indoleacetic, and indolepropionic. In the case of indolepropionic acid the observable initial development of the fruit was much slower than that in the case of pollination, and markedly slower than any of the other compounds.

Following pollination, approximate mature size of holly fruits is attained in seven or eight weeks. To all outward appearances the parthenocarpic fruits are like those developed following pollination, with the exception that, in the case of naphthaleneacetic acid, the fruits continue to be much greener in color and somewhat larger in



size (fig. 2). Data on the ripening of the fruits and the development of the red color following chilling weather is still to be obtained.

ABSCISSION RELATED TO SECOND GROWTH.—It was mentioned earlier that with bearing holly trees only one flush of growth normally occurs. With young seedlings and rooted cuttings, however, a second and even third flush is not uncommon if conditions for growth are very favorable prior to maturation of the terminal bud. This



FIG. 2.—Left: pistillate plant eight weeks after pollination. Right: similar plant sprayed with 0.01 per cent naphthaleneacetic acid.

second flush of growth, amounting to as much as 5-10 inches, occurred in many of the experimental plants, both in the pollinated and the sprayed lots (fig. 3). The number of sprayed plants making a second growth differed with the growth substance used and with the concentration. The data in table 3, showing the percentages of plants making this growth, are based on all concentrations applied and without regard to the set of fruit. They are not strictly comparable, therefore, since the concentrations of all four compounds

are not in identical ranges. The percentage of all fruits to drop is, however, closely associated with the percentage of all plants making second growth. Every sprayed plant which made a second growth



FIG. 3.—Left: pollinated plant which made a second flush of growth but held its fruit. Right: plant sprayed with indoleacetic acid which had eight fruits which abscised when second flush of growth occurred. Approximate upper half of entire stem in each plant is of second flush growth.

dropped all its fruit. On the other hand, none of the pollinated plants dropped their fruit although all made a second growth. This

lack of abscission in the case of the pollinated fruits is probably associated with the development of the embryos within their seeds. Embryos are not present in the parthenocarpic fruits.

The inhibiting effect of the sprays on second growth was most marked in the case of naphthaleneacetic and indolebutyric acids in the higher concentrations used. As apparent from table 3, indolepropionic acid had very little influence in this respect.

FRUITS SET BY APPLYING INDOLEACETIC ACID TO SOIL.—In addition to parthenocarpy obtained by spraying, fruits were also produced by watering the soil around the roots of holly plants with rela-

TABLE 3  
FRUIT ABSCISSION AND SECOND GROWTH OF HOLLY

TREATMENT	PERCENTAGE FRUITS DROPPED	PERCENTAGE PLANTS MAKING SECOND GROWTH
Naphthaleneacetic. . . . .	25.5	36.3
Indoleacetic. . . . .	40.4	56.8
Indolebutyric. . . . .	51.8	53.5
Indolepropionic. . . . .	95.7	87.5
Pollinated. . . . .	0.0	100.0

tively concentrated solutions of indoleacetic acid during full bloom. A 0.15 per cent solution added in sufficient amounts on two successive waterings so that considerable drainage from the pots took place caused a number of flowers to set fruit. Relatively few plants were used in this experiment and no record was secured of the percentage set. Indoleacetic acid was the only compound applied in this way. The first trials with concentrations ranging from 0.0025 to 0.02 per cent were without effect. A concentration of 0.15 per cent produced no apparent injury and no epinasty.

Fruit was also set on some plants with indoleacetic acid by introducing small quantities of the dry powder into holes in the stem made with a small nail.

PARTHENOCARPIC STRAWBERRIES.—Individual potted plants of a strictly pistillate strawberry selection, protected in the greenhouse against chance pollination, were sprayed during a portion of their

blooming period with indoleacetic acid in concentrations of 0.1, 0.05, 0.025, 0.01, and 0.005 per cent respectively. All of these concentrations resulted in many of the blossoms producing apparently normal achenes which, upon subsequent examination, proved to be devoid of embryos. With the 0.05 and 0.1 per cent concentrations a number of the receptacles developed and ripened into apparently normal fruits (fig. 4). Never more than one fruit of an inflorescence, however, developed completely. In the lower concentrations the receptacles made only a slight initial growth, which soon ceased, although achenes usually developed. The receptacles or achenes of unsprayed flowers made no development.

Although some of the parthenocarpic achenes were cut open for examination, the majority, several hundred in all, were planted to ascertain whether any viable embryos were present. Only one rather weak seedling was obtained, which is still alive and is to be examined later for chromosome abnormalities. Achenes from pollinated fruits of the same strawberry selection germinated freely.

EXPERIMENTS WITH APPLE AND GRAPE.—Orchard trees of the Starking apple, a self-sterile variety, were protected from cross pollination and sprayed, when in full bloom, with solutions of indoleacetic acid ranging in concentrations from 0.01 to 0.06 per cent, but no fruits developed. In the higher concentrations used, marked injury to the pistils occurred, and the negative results were therefore not attributed to insufficient concentration, but tentatively to the relatively long style of the apple, through which length the stimulus of the growth substance might have some difficulty in passing. No attempts were made with the styles shortened by cutting.

The Brighton grape, which is self unfruitful, likewise failed to respond to naphthaleneacetic acid in concentrations ranging from 0.0005 to 0.01 per cent. This range was arbitrarily selected, and having failed, there was no opportunity to try stronger concentrations since the flowering period had passed. Since no serious injury occurred at the highest concentration used, it is thought that a still stronger solution might have resulted in parthenocarpy. The grape, like the holly, has a very short style.

It is evident from the results reported here that not all plants can



FIG. 4.—Parthenocarpic strawberry fruits. Upper: flowers sprayed with 0.1 per cent indoleacetic acid, which also caused lengthening and twisting of pedicels. Lower: fully developed parthenocarpic fruit of good size. Achenes which appear normal are empty. Note that never more than one fruit per inflorescence develops.

be expected to respond as readily as the holly to spraying with these compounds. Entirely apart from scientific interest, however, the results with holly do constitute an example of the practical value of the use of growth promoting compounds in effecting fruit setting.

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# EFFECTS OF SUDDEN CHANGES OF TEMPERATURE ON ELONGATION RATE OF PRIMARY ROOT

LAURETTA E. FOX

(WITH ONE FIGURE)

## Introduction

ASKENASY (1), in a study of the effects of temperature upon growth, found that the roots of maize seedlings did not elongate at temperatures of 3° to 9° C. If roots which had been exposed to these low temperatures were returned to 20° C., they usually elongated more slowly than the controls for several days. ASKENASY noted that sudden changes of temperature produced a different effect from gradual changes. LEHENBAUER (8) gave data which supported this conclusion. The writer began an investigation of the effects of sudden changes of temperature on the elongation rate of roots under the direction of Professor CHARLES F. HOTTES at the University of Illinois. This paper reports the results of a continuation of this study.

## Material and methods

Seedlings of *Phaseolus vulgaris*, Burpee's stringless greenpod bean, were used. The seeds were planted on moist, porous clay blocks in galvanized iron germinating pans. As soon as the seeds germinated the seedlings were removed, and all seedlings which did not possess straight roots and those growing from embryos broken in milling were discarded. The seeds were placed in baskets with the primary root extending into a pan of tap water. A pan of similar dimensions was inverted over the plants. The seedlings were grown in the constant temperature cases and kept at a uniform temperature.

As soon as a majority of the primary roots were 6 cm. long, their length was measured with a millimeter ruler and the length of each root recorded. Each seedling was labeled. After all the seedlings of a group were measured and labeled the roots of the seedlings were exposed suddenly to low temperatures.

A refrigerating unit was used for chilling. Large test tubes, filled with tap water, were extended into the brine. The roots were ex-

tended through perforated corks in the necks of the test tubes, about 1 cm. of the root tip extending into the cold water. The same type of result was obtained when the entire primary root extended into the water. A glass was inverted over the stems and leaves to prevent excessive transpiration. The roots were chilled for a definite period of time, and the temperature of the water in the test tubes was recorded with a maximum-minimum thermometer.

When seedlings were removed after chilling, they were placed in water of the temperature at which they were growing before chilling, and their growth was continued in the temperature case. At 24 or 48 hour intervals the lengths of the roots were measured. All measurements were taken at 8 A.M. The tap water in the pans was changed each time the roots were measured.

### Experimental data

#### EFFECT OF PERIOD OF CHILLING UPON ELONGATION RATE OF ROOT

Bean seedlings grown at 20° C. were sorted into groups of twenty-five having uniform range of length and elongation rate (for 24 hours before chilling). The rate of elongation for each group for the 24 hour period before chilling was 0.72-0.76 cm. / 24 hours / root. These groups were exposed to low temperatures 24 hours before the primary roots reached the first period of greatest elongation in the S-shaped curve of elongation. The roots were chilled at 9 A.M. to 3° C. for varying periods of time. Some of the roots were chilled for 3 minutes; some for 10 and 20 minutes; and others for 30, 40, 50, 60, 70, 80, 90, and 120 minutes. It was found that chilling for 3 minutes produced an accelerated elongation rate of the root during the following 24 hours (fig. 1). During the following 24 hour period the elongation rate of these roots was low. Roots which were chilled for 10 minutes were retarded in their elongation rate during the first 24 hour period after chilling. During the fifth 24 hour period after chilling they elongated slightly more rapidly than did the roots of the control seedlings. Those chilled 20, 30, 40, and 50 minutes elongated less rapidly than did the control roots.

The roots chilled 70 minutes elongated more rapidly than those chilled 10 or 50 minutes. Numerous other experiments confirmed this fact. Chilling a longer period of time must produce some ad-



justment in the protoplasm of the root. After this change has taken place the root can elongate more rapidly when transferred to  $20^{\circ}\text{C}$ . No root chilled 70 minutes elongated as rapidly as the roots of the control seedlings.

The roots chilled 90 minutes elongated less rapidly than those chilled 70 minutes. Those chilled 120 minutes elongated more rap-

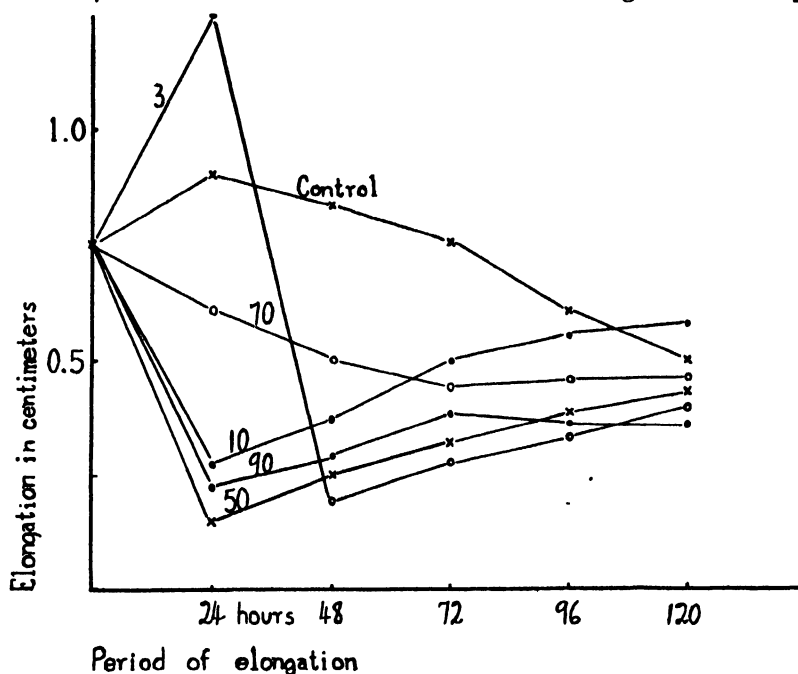


FIG. 1.—Effect of period of time roots are chilled on their elongation rate when returned to  $20^{\circ}\text{C}$ . Seedlings grown at  $20^{\circ}\text{C}$ .; roots chilled to  $3^{\circ}\text{C}$ . for various periods of time and again grown at  $20^{\circ}\text{C}$ . Hours after chilling are plotted along abscissa; increase in length for each 24 hour period after chilling is recorded along ordinate. Number of minutes each group was chilled shown on the curve for each group.

idly than those chilled 90 minutes, but not so rapidly as those chilled 70 minutes. The nature of the adjustment which must be made by these cells has not yet been studied.

#### EFFECT OF TIME OF DAY ROOTS ARE CHILLED ON ELONGATION RATE

Bean seedlings were grown at  $20^{\circ}\text{C}$ . and the primary roots exposed to  $3^{\circ}\text{C}$ . for 10, 20, 30, and 40 minutes. The roots of twenty-

five seedlings were chilled at 8 A.M. for 10 minutes. The same number were chilled at 8 A.M. for 20, 30, and 40 minutes. Similar groups were chilled at 9 A.M., 10:30 A.M., 12:10 P.M., 1 P.M., and 3 P.M. These seedlings were placed under uniform conditions in the temperature case maintained at 20° C. There were some control seedlings in each pan. Measurements were taken at 48 hour intervals.

The roots chilled at 8 A.M. elongated about as rapidly as did the roots of the control seedlings during the following 48 hour period (table 1). The roots chilled at 9 A.M. elongated much more slowly during the first 48 hour period after chilling than those chilled at 8 A.M. The roots chilled at 10:30 A.M. elongated more rapidly than those chilled at 9 A.M. The elongation rate of those chilled at 12:10 was greater than that of those chilled at 10:30. The elongation rate of those chilled at 1 and at 3 P.M. was less than that of those chilled at 12:10 P.M.

Numerous experiments gave similar results. In each case the roots chilled at 9 A.M. and at 1 P.M. or 3 P.M. showed a retarded elongation rate. Those chilled at 8 A.M. or about 12 noon showed less retardation in elongation rate.

#### PERIODICITY OF MITOSIS IN BEAN ROOT TIPS

KELLCOTT (6) found that elongation and mitotic activity in onion root tips grown at 20° C. show periodicity. Elongation was at its maximum when mitotic activity was at its minimum, and vice versa. The periods of greater mitotic activity in the onion root tips studied were at 11 and 1 P.M. KARSTEN (5), working with *Vicia faba*, *Zea mays*, and *Pisum sativum*, concluded that roots have no periodicity of mitotic activity, but that stems show periodicity to a marked degree. LAUGHLIN (7) studied the periodicity of mitosis in onion root tips. He concluded that mitosis does show periodicity.

The root tips of seedlings grown at 25° C. were collected in formalin-acetic-alcohol solution (3). The root tip was dissected out with a small needle. The material thus dissected was stained as a smear with iron aceto-carmin stain (3). The number of cells in the various stages of mitosis were counted under an oil immersion lens. The number of cells in resting or vegetative stage, prophase, metaphase, anaphase, and telophase are recorded in table 2. The number after

TABLE 1  
 ELONGATION RATE OF ROOTS CHILLED AT DIFFERENT  
 HOURS OF THE DAY

HOUR CHILLED	DURATION OF CHILLING	INITIAL LENGTH OF ROOT (CM.)	ELONGATION RATE (IN CM.) DURING	
			FIRST 48 HOURS AFTER CHILLING	SECOND 48 HOURS AFTER CHILLING
8 A.M.	Control. . . . .	av. * 6.1 r. * 5.0-7.5	1.4 1.0-1.6	2.0 1.6-2.4
	10 minutes. . . .	av. 6.0 r. 4.6-8.0	1.2 1.0-1.3	2.1 1.5-2.7
	20 minutes. . . .	av. 6.0 r. 5.1-8.0	1.0 0.7-1.5	1.6 1.4-2.0
	30 minutes. . . .	av. 5.9 r. 4.8-6.8	1.3 1.2-3.1	1.9 1.7-2.8
	40 minutes. . . .	av. 6.0 r. 4.6-7.9	1.2 0.9-1.4	2.0 1.3-1-8
9 A.M.	Control. . . . .	av. 6.0 r. 5.0-8.2	1.6 1.4-1.8	1.9 1.7-2.0
	10 minutes. . . .	av. 6.2 r. 6.1-7.3	0.5 0.6-1.1	0.8 0.0-1.8
	20 minutes. . . .	av. 5.8 r. 4.5-8.1	0.6 0.0-0.9	0.8 0.2-1.2
	30 minutes. . . .	av. 6.2 r. 4.9-8.2	0.4 0.2-0.8	0.9 0.0-1.7
	40 minutes. . . .	av. 6.1 r. 5.6-6.9	0.4 0.0-1.4	0.8 0.2-1.8
10:30 A.M.	Control. . . . .	av. 6.0 r. 5.0-7.1	1.3 1.1-1.7	2.1 1.7-2.6
	10 minutes. . . .	av. 6.0 r. 5.0-7.1	0.8 0.5-1.2	2.0 1.1-2.6
	20 minutes. . . .	av. 6.0 r. 4.7-8.0	0.9 0.6-1.2	1.5 0.1-2.4
	30 minutes. . . .	av. 5.7 r. 5.0-6.6	0.9 0.7-1.2	1.5 1.3-1.9
	40 minutes. . . .	av. 6.3 r. 4.6-7.8	1.2 1.0-1.4	1.9 1.4-2.3

\* Average and range for 25 roots.

TABLE 1—*Continued*

Hour chilled	Duration of chilling	Initial length of root (cm.)	Elongation rate (in cm.) during	
			First 48 hours after chilling	Second 48 hours after chilling
12:10 P.M.	Control. . .	av. 6.1 r. 4.5-6.7	1.3 1.0-1.8	1.9 1.3-2.5
	10 minutes .	av. 6.4 r. 5.0-7.9	1.4 0.9-1.7	2.2 1.5-2.8
	20 minutes .	av. 5.8 r. 5.0-7.5	1.2 1.0-1.4	2.2 2.0-2.3
	30 minutes .	av. 6.0 r. 5.5-7.2	1.2 1.0-1.4	1.5 0.0-2.1
	40 minutes .	av. 6.3 r. 4.5-7.5	1.0 0.7-1.5	2.2 1.7-3.0
1 P.M.	Control. . . .	av. 6.3 r. 5.2-6.8	1.4 1.0-1.8	2.0 1.5-2.7
	10 minutes .	av. 6.6 r. 5.5-8.1	0.4 0.2-0.6	0.4 0.0-0.9
	20 minutes .	av. 5.9 r. 5.0-6.5	0.6 0.2-0.8	0.6 0.0-1.1
	30 minutes .	av. 6.3 r. 4.6-7.3	0.5 0.3-0.7	0.5 0.0-1.4
	40 minutes .	av. 5.9 r. 4.7-7.4	0.7 0.2-0.9	0.8 0.2-1.6
3 P.M.	Control. . . .	av. 6.0 r. 4.6-7.1	1.5 1.0-1.8	2.0 1.7-2.8
	10 minutes . .	av. 6.3 r. 5.1-7.2	0.6 0.2-0.8	0.9 0.6-1.6
	20 minutes . . .	av. 6.2 r. 4.9-7.6	0.8 0.0-1.0	0.8 0.4-1.6
	30 minutes . . .	av. 6.0 r. 5.2-7.3	1.0 0.7-1.3	1.2 0.6-1.4
	40 minutes . . .	av. 5.9 r. 5.0-6.8	0.6 0.2-1.2	0.7 0.0-2.0

each stage indicates the equivalent of the stages counted by LAUGHLIN (7). There is little difference between the number of cells in mitosis at the various hours of the day. There are more cells in prophase at 9 than at 10 or 11 A.M. There are more cells in prophase again at 12 noon than at 1 P.M. The total number of prophases is greater at 9 A.M. than at 12 noon or 3 P.M. There are more cells in anaphase and telophase at 11 A.M. and at 2 P.M. than at other hours of the day.

The primary roots of seedlings of these groups were chilled to 3° C. for 30 minutes at 8, 9, 10, 11 A.M., 12 noon, 1, 2, and 3 P.M.

TABLE 2  
NUMBER OF CELLS IN MITOSIS

TIME	STAGE IN MITOSIS (EQUIVALENT OF LAUGHLIN'S STAGES DESIGNATED BY NUMBER AFTER STAGE)									TOTAL NUMBER IN MI- TOSIS
	REST- ING	EARLY (1, 2) PRO- PHASE	MIDDLE (3) PRO- PHASE	LATE (4) PRO- PHASE	META- PHASE (5)	ANA- PHASE (6, 7)	LATE (8) ANA- PHASE	EARLY (9) TELO- PHASE	LATE (10) TELO- PHASE	
8 A.M. ....	907	11	3	3	23	11	17	8	17	93
9 ..... 867	41	23	16	21	13	3	14	2	133	
10 ..... 894	32	10	10	16	16	10	11	1	106	
11 ..... 894	1	2	5	19	24	15	6	34	106	
12 M. .... 879	59	23	0	19	8	9	2	1	121	
1 P.M. .... 918	19	20	0	17	3	6	5	12	82	
2 ..... 905	8	4	8	10	14	13	7	31	95	
3 ..... 901	18	12	0	30	13	11	14	1	89	

They were chilled 48 hours before reaching the first period of greatest elongation in the S-shaped curve of growth, and then grown at 25° C. The rate of elongation of the primary roots of these seedlings is recorded in table 3. Roots chilled at 9 A.M., 12 noon, and 3 P.M. showed more retardation than those chilled at 8 and 11 A.M., or 2 P.M. It was found that the roots which showed a greater retardation of elongation rate after chilling were those which had been chilled when more cells were in prophase. ATKINSON (2) reported that pollen mother cells obtained during cold days in early spring were in prophase. No stages of division beyond prophase were found. SAX (10) found that nuclear and cell divisions in pollen mother cells and microspores of *Tradescantia* were disturbed by exposure to low and high temperatures.

TABLE 3  
ELONGATION RATE OF ROOTS CHILLED AT  
DIFFERENT HOURS OF THE DAY

HOUR CHILLED	DURATION OF CHILLING	INITIAL LENGTH OF ROOT (CM.)	ELONGATION RATE (IN CM.) DURING	
			FIRST 48 HOURS AFTER CHILLING	SECOND 48 HOURS AFTER CHILLING
8 A.M.	Control . . . . .	av. * 6.2 r. * 5.1-7.5	2.6 1.7-3.0	3.1 2.0-3.6
	30 minutes . . . .	av. 6.0 r. 5.1-7.7	2.1 1.4-3.0	2.7 1.8-2.8
9	Control . . . . .	av. 6.0 r. 5.0-7.1	2.8 1.6-2.8	3.0 1.8-3.2
	30 minutes . . . .	av. 6.1 r. 5.6-7.1	1.0 0.5-2.1	1.8 0.0-3.6
10	Control . . . . .	av. 6.1 r. 5.6-6.8	2.4 1.7-3.2	2.9 1.9-3.4
	30 minutes . . . .	av. 6.0 r. 4.6-7.2	1.7 1.1-2.0	2.8 1.0-3.5
11	Control . . . . .	av. 5.8 r. 5.0-6.5	2.5 1.8-2.8	2.9 2.1-3.3
	30 minutes . . . .	av. 6.1 r. 5.1-7.2	2.7 1.5-3.0	3.0 2.1-3.4
12 NOON	Control . . . . .	av. 5.9 r. 4.9-6.9	2.5 1.5-2.9	2.9 1.6-3.1
	30 minutes . . . .	av. 6.0 r. 4.8-6.7	1.0 0.2-1.2	1.2 0.0-2.1
1 P.M.	Control . . . . .	av. 5.8 r. 5.0-7.9	2.4 1.6-3.1	2.9 1.8-3.6
	30 minutes . . . .	av. 5.9 r. 5.0-7.1	1.3 1.0-1.5	2.0 1.5-2.4
2	Control . . . . .	av. 6.1 r. 5.1-7.2	2.5 1.5-2.8	2.8 1.9-3.4
	30 minutes . . . .	av. 6.0 r. 4.7-6.8	2.0 1.6-2.5	2.5 1.7-3.1
3	Control . . . . .	av. 6.2 r. 5.2-6.8	2.3 1.6-3.1	2.8 1.4-3.7
	30 minutes . . . .	av. 6.1 r. 5.1-6.9	0.9 0.0-1.2	1.2 0.5-2.0

\* Average and range for 20 roots.

It was noted that the turgidity of the cells varied in relation to the hour of the day the roots were chilled. Some roots were more turgid when removed from chilling than were other roots. Investigations of this relationship will be reported later. There are many factors which vary in cells with the mitotic cycle.

#### EFFECT OF HEATING ON ELONGATION RATE OF ROOTS

Seedlings were grown at 15° C. and their roots exposed to water maintained at 35° or 40° C. for 30 minutes. The seedlings were re-

TABLE 4  
EFFECT OF INITIAL LENGTH AND AGE OF ROOTS ON ELONGATION  
RATE OF ROOTS AFTER CHILLING

ROOTS CHILLED AT 3°C. FOR 20 MINUTES; SEEDLINGS GROWN AT 20°C.					
AGE (DAYS SINCE PLACED IN TEMPERATURE CASE)	INITIAL LENGTH (CM.)	AVERAGE ELONGATION FOR 48 HOURS BEFORE CHILLING (CM.)*	HOUR CHILLED	AVERAGE ELONGATION FOR FIRST 48 HOURS AFTER CHILLING (CM.)*	AVERAGE ELONGATION FOR SECOND 48 HOURS AFTER CHILLING (CM.)*
6 . . . . .	4.5-5.0	1.9	9 A.M. 12 NOON	0.5 2.0	0.6 1.4
6 . . . . .	7.0-8.5	2.2	9 A.M. 12 NOON	0.8 2.6	0.7 1.5
8 . . . . .	5.5-6.7	1.1	9 A.M. 12 NOON	0.1 1.2	0.3 1.1
8 . . . . .	8.0-9.0	1.5	9 A.M. 12 NOON	0.8 1.4	1.0 1.2

\* Average elongation for 20 roots recorded.

turned to 15° C. and their elongation measured at regular intervals. Similar results to those reported for roots which were chilled were obtained. More marked results were produced by treating at 40° than at 35° C.

#### EFFECT OF AGE OF SEEDLINGS AND THEIR INITIAL LENGTH ON ELONGATION RATE AFTER CHILLING

The initial length of the seedlings did not affect the type of response of the seedlings after chilling. The stage of elongation in the S-shaped curve of growth had little effect on the response to chilling. These data are recorded in table 4.

### Discussion

Numerous investigators (12, 6, 4, 5, 7) have noted a rhythm in mitosis. KELLICOTT found that elongation and mitosis were periodic. The period of greater mitotic activity was the period of less elongation, and vice versa. When a cell is undergoing mitosis it is resting from its normal metabolic activities, and when it is metabolically active it does not undergo mitosis. The amount of food stored in the cell, the rate at which food is used, the oxygen consumption, and the water absorption must vary during the different stages of this growth cycle of the cell. Viscosity of the protoplasm is lowered during the early stages of mitosis. It rises during the later stages, and then drops to its original value (11). The turgor pressure also varies. In animal cells it has been found that reversible changes in surface tension, electrical polarization, permeability, and osmotic pressure take place during mitosis. The susceptibility of sea urchin eggs to heat and poisons is greater during mitosis. The period of greatest sensitivity is prophase. The sensitivity to poisons is less 35-45 minutes after cleavage (9).

At the present time it is impossible to state which factors are responsible for the results obtained. The data seem sufficient to conclude that the hour of day roots are exposed to low temperatures influences their elongation rate when transferred to 20° C. In control seedlings it was found that cells produced by mitotic activity started to elongate within the first 24 hours after their formation. If the mitotic activity of the embryonic cells of the root tip was disturbed by exposure to low temperatures, elongation during the following periods would be slower. Other changes, which occur periodically in the cells, may also influence the elongation rate after exposure to low temperatures.

### Summary

1. The period of time roots were exposed to low temperatures influenced their elongation rate when returned to 20° C. After two or three 24 hour periods the elongation rate tended to return to the elongation rate of the controls.

2. The hour of the day at which root tips were exposed to low temperatures determined the elongation rate of the roots when trans-



ferred to 25° C. The roots chilled when more cells were in prophase were retarded in elongation rate. Cells formed by mitotic activity of the embryonic cells started to elongate within the first 24 hours after their formation. Other factors may influence the results obtained.

3. Exposing roots grown at 15° C. to high temperatures produced results similar to those produced in roots exposed to low temperatures.

4. The age of roots and their initial length did not affect the elongation rate of the roots which had been exposed to low temperatures.

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## CURRENT LITERATURE

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*Phytohormones*. By F. W. WENT and KENNETH V. THIMANN. New York: Macmillan Co., 1937. Pp. xi+294. 62 figs. \$4.00.

The tone of this work is well expressed by the writers when they state, in relation to the question of priority of the discovery of the auxins, "We feel that the gradual unfolding of the current conceptions of the cooperation of different workers has made it impossible to credit any one person with such a discovery, and it is to be hoped that the reader of this book will gain the impression of a steady and collective advance rather than of individual contributions." And again, "There is danger, in any rapidly developing field, of an accumulation of unclassified facts and unproven theories which makes further development much less certain. In the field of phytohormones this is particularly unjustifiable, because the experimental procedure is relatively simple and the equipment necessary is not too elaborate. If the criteria of high-class experimental work continue to be observed, then we may look forward in the next few years to the rapid solution of a great many of the interesting problems of growth and development." Indeed, those who wish to follow the development of this interesting field may well applaud these expressed desires of the authors and trust that the present torrential rush toward priority, and above all, the formulation of theories and interpretation on less than meager data, may be slackened.

The details given in the chapter on the technique of auxin determination are direct and helpful without over-simplification of the process. The following six chapters embody an enumeration of a wide variety of experiments reported by many investigators, which deal with the formation and occurrence of auxins, their chemistry, their transport in the plant, and suggested correlations and interpretation of physiological processes and responses of plants on the basis of the phytohormone concept. Here are both the most and the least convincing parts of the book, for one feels strongly the great need of additional evidence, particularly such as would deal with the interrelationships of foods, nutrients, and hormones both from a qualitative and a quantitative viewpoint. Needed also is histological and cytological evidence. That presented seems extremely meager compared with the abundant references on generalized or gross responses. All these deficiencies, however, are in the field of phytohormone study. The authors could do no more than make these lacks obvious.

There is a comprehensive list of references to pertinent literature and helpful indexes to authors and subject matter. The book, assembled and written by two of the most active contributors to the field of study of phytohormones, has

directness of attack and frankness of analysis, both in relation to methodology and to interpretation of the results of experimentation. This treatise may well serve as one means toward stabilization of the subject.—E. J. KRAUS.

*Studies on Wheat Grown under Constant Conditions. A Monograph on Growth.*

By H. VAN de SANDE-BAKHUYZEN. Stanford University, Food Research Institute, 1937. Pp. xvi+400. Figs. 27. \$4.00.

A fundamental study of the growth of the wheat plant under controlled conditions has been made under the auspices of the Food Research Institute, and this, in many respects excellent, monograph presents the results of the investigation. There are thirty-six chapters grouped into seven main sections, which follow an introductory chapter. These seven sections bear the following titles: the growth curve in annual plants; methods and materials; general growth features; dry weight and moisture content of the different organs; dry weight and moisture of the standard plant; nitrogen and carbon of the organs; and nitrogen metabolism in relation to growth and development. In the collection of data, and in the interpretation of the materials of section VI, the author had the aid of E. P. GRIFFING and CARL L. ALSBERG. Summaries at the end of the various sections make it easy to get the gist of the report with a minimum effort.

Some of the information presented has been drawn from previously published investigations. The chapters on nitrogen balance, on the relation between respiration, nitrogen metabolism, and growth, and on aging in respect to external conditions are especially interesting.

In general, a very satisfactory beginning has been made in understanding the growth processes of a single important food plant. Naturally any work of this kind always leaves the impression that we still need much more information than the monograph can supply. It is a satisfaction to know that intensive and extensive research of this kind can be continued indefinitely, so that there will be an ever increasing knowledge of the nature of growth processes. All of the other prominent food plants deserve the same systematic investigation of their growth processes and responses.—C. A. SHULL.

*Soil Conditions and Plant Growth.* By SIR E. J. RUSSELL. London: Longmans, Green and Co., 1937. Pp. viii+655. Figs. 65. 21/0 net.

With only slight increase in size, the seventh edition of this masterpiece is now available. The author has incorporated much new material into the text, as the result of his visits to many European Agricultural Experiment Stations and his contacts with soil scientists who attended the Third International Congress of Soil Science at Oxford in 1935. The appendix of analytical methods has been omitted, and the bibliography shortened to conserve space. The volume is without doubt the most valuable single book on this subject in the English language.—C. A. SHULL.

# THE BOTANICAL GAZETTE

December 1937

## VEGETATION OF CERTAIN SAND PLAINS OF CONNECTICUT<sup>1</sup>

CHARLES E. OLMSTED

(WITH TWENTY-SIX FIGURES)

### Introduction

Extensive, nearly level plains of sandy stratified drift are common features in the central lowland of Connecticut. Terraces with sands of fine texture are utilized in agriculture, primarily in the growth of tobacco or truck crops, but deposits of coarser texture are of little or no agricultural value. When not used for urban or industrial purposes, they are covered with various xerophytic types of natural or semi-natural plant communities, such as lichen-grassland, dominated by *Cladonia* spp. and *Andropogon scoparius*;<sup>2</sup> scrub, with various species of shrubs and low trees; and woodlands and low forests of pitch pine, black oak, grey birch, red cedar, etc. In extreme

<sup>1</sup> This dissertation, modified for publication, was presented for the degree of Doctor of Philosophy in Yale University in 1936. The work was supported in part by a Sterling Fellowship in the Graduate School of Yale University, during the academic year 1932-33.

<sup>2</sup> With minor exceptions, the nomenclature used is that of the following authorities: *Cladonia*, EVANS (22, 23, 24); other lichens, EVANS and MEYROWITZ (25); mosses, EVANS and NICHOLS (26); grasses, HITCHCOCK (34); trees, SUDWORTH (52); other vascular plants, GRAVES, EAMES, *et al.* (30) and HARGER, GRAVES, *et al.* (32). The following species and varieties, collected from North Haven or Wallingford, are not recorded from these towns, or are not listed for Connecticut in the last two publications cited: *Panicum auburne* Ashe (not *P. albemarlense* Ashe); *Carex merritt-fernaldii* Mackenzie; *C. umbellata* Schkuhr, var. *tonsa* Fernald; *C. brevior* (Dewey) Mackenzie; *Helianthemum propinquum* Bickn.; *Stachys hyssopifolia* Michx.; *Eupatorium hyssopifolium* L.; and *Aster spectabilis* Ait.

cases the vegetation may be so sparse that the aspect is desert-like. Such areas are designated as barrens.

One of the most noteworthy of these terraces extends along the east side of the Quinnipiac River in the towns of North Haven and Wallingford, for a distance of 15 to 16 miles, between New Haven and Meriden, Connecticut, with an average width of 1 to 1½ miles. Numerous barren areas are found on this series of plains. Their anomalous occurrence in a deciduous forest climate, with an average precipitation of 45 inches a year, has interested numerous scientists. In the main the ecological relations have been inadequately understood, and the explanations advanced are far from complete or are seriously in error. No less interesting are the closed plant communities of diverse types, with their numerous transitions. Among these, lichen-grassland communities are of greatest extent and interest. The object of the present investigation was to describe these various types of vegetation, and to provide a more nearly complete explanation of their occurrence on the basis of correlation with various ecological factors.

### Review of previous work

BRITTON (8), BOWMAN (5), NICHOLS (42, 43), BROWN (10), MORGAN (41), and FLINT (27) have made suggestions to account for the xerophytic vegetation of these sand plains, and for failure of crop plants on them. The barren areas have attracted most attention and it has been assumed that they might represent a condition not modified by man. Their persistence has been attributed variously to almost total absence of humus, continual drying up of the surface, intense heat on sunny days, wind sweep and shifting character of the surface, nitrogen deficiency, high rate of evaporation, low water table, excessive looseness and porosity of the sandy soil, and low water retention. BRITTON (8) thought the scattered occurrence of black oaks on the barrens was due to burial of acorns by squirrels.

The explanations of these workers seem either incomplete in that historical factors are completely ignored, or in partial error in attributing too much importance to the soil moisture relationship. These facts have influenced the course of the present investigation. Methods used are presented with results obtained in subsequent sections.

## Regional habitat conditions

### CLIMATE

General climatic conditions should resemble closely those of New Haven, and data are given for that city,<sup>3</sup> based on a 63-year record. Temperatures are given in Fahrenheit and precipitation in inches.

The annual mean temperature ranges from 47.1° to 53.1°, with an average of 50°. January, February, and July means are 28.7°, 28.6°, and 72.2° respectively. Absolute maximum and minimum temperatures recorded are 101° in July and -15° in February. The average length of the growing season between killing frosts is about 190 days, from April 15 to October 23, with minimum and maximum of 142 and 226 days respectively. The average annual precipitation is 45.79, with maximum and minimum of 60.26 and 34.72. June shows the lowest monthly precipitation average with 3.19, while August is highest with 4.39. Lowest and highest monthly precipitation extremes in the 63 years are 0.17 and 17.08, for September and July respectively. Amounts under 1 inch are recorded for all months except January, April, and July, while records just at or below 0.25 are found for March, May, June, August, September, and October, indicating occasional conditions of severe stress in the water relations of plants during the growing season. Yearly sunshine is 60 per cent of the possible amount, and July is high with 64 per cent. Seasonal snowfall is 39.3 inches, with minimum and maximum of 7.3 and 76.0.

### PHYSIOGRAPHY

The areas of detailed study lie between the village of North Haven (lower left corner of figure 3) and the borough of Wallingford (upper right corner of figure 1) within the Quinnipiac-Farmington lowland of the larger Connecticut Valley lowland. FLINT (27, 28) has described in detail the probable time and manner of origin of the nearly level terraces of coarse sandy stratified drift which are found throughout this lowland.

The upper surface of the terrace studied slopes southward from an 80 foot level at Wallingford to 40 feet above sea level at North

<sup>3</sup> Data from the Annual Meteorological Summary, 1935, of the New Haven Office of the U.S. Weather Bureau.

Haven. Its western margin meets the swampy floodplain or lower sandy terraces of the Quinnipiac River through a steep, west-facing slope, 10 to 20 feet or more in height. A number of springs emerge at the base of this slope, indicating underlying clay beds, but their occurrence is not a reliable criterion of the depth of the ground water table nor of the thickness of the sand deposit in the terrace as a whole. BROWN (10) states that the depth of stratified drift in North Haven in many places may be as much as 50 feet, and that the maximum depth is probably more than 100 feet. His data show that the depth to water in dug wells located on the terrace in North Haven varies from 2 to 27 feet, lying in the majority of the wells between 10 and 20 feet. The average depth in Wallingford is probably somewhat greater. Certain areas on the terrace do show a ground water table above or near the surface during winter and spring, especially near the eastern margin, which adjoins ridges of Triassic sandstone covered with a thin layer of till. Perhaps this high water table is caused by the presence of collecting basins in the underlying and uneven sandstone or till. Till deposits project above the level surface of the plain locally, forming small rounded mounds or ridges, so that variation in the depth of ground water from place to place might be expected. But sandpit excavations, soil pits, and other lines of evidence indicate that in the areas of detailed study the vegetation is never under the influence of a high water table, and that the coarse sand deposit extends many feet in depth.

In addition to the till mounds, the continuity of the plain is broken by a number of small transverse streams which have cut through the sand to considerable depth. Some of these have widened their ravines and now have small floodplains, and the vegetation under their control is decidedly different from that of the terraces. More important in the present problem is the occurrence of a number of wind-formed sand ridges and hummocks. The largest of these reach lengths of several hundred feet, widths of 50 feet or more, and heights of from 3 to 10 feet. They stand isolated from one another in the surrounding plain. The long straight axes extend for the most part from east to west or from southwest to northeast. North and northwest winds have been most important in their formation. The relatively recent origin of these ridges, which may justifiably be

called dunes, will be brought out later. In general, dunes are of rare occurrence on the inland terraces in Connecticut. FLINT (27) pointed out that "the well developed soil at the surface of each terrace may date from the time when the terrace was exposed by draining, and when therefore ice was still present. If so, vegetation appeared on the terraces *pari passu* with the exposure of the latter to the atmosphere. That this took place is rendered likely by the poor development of loess and dunes even where the exposed terraces were most extensive."

### SOILS

All of the soils of Connecticut were mapped by MARBUT (39) in the gray-brown podzolic group, but with imperfectly developed profiles. The soils of the terrace in question were mapped by MORGAN (41) as Merrimac coarse sand. Some of them belong to finer types in this same series, while others may be referred to the Hartford series, or to transitions. According to MORGAN both series are well drained, with loose, coarse, gravelly, and sandy substrata at from 24 to 36 inches' depth, derived from level or nearly level terraces of sand and gravel deposited at the close of the glacial epoch. A color difference, probably caused by the presence of limonite in the Merrimac series and of hematite in the Hartford series, is the most obvious difference between the two series. The rock particles in each series are chiefly mineral fragments of quartz and feldspar with hornblende, micas, garnet, and magnetite as lesser and more variable constituents, all more or less stained by hematite or limonite. The acidic nature is obvious, but it is true that these terrace deposits do not differ greatly in their total chemical composition from the average for Connecticut rocks. Most of the soils in the area of study may be referred to the Merrimac series, and most of them are coarse sands.

MARBUT (39) said of the Merrimac soils that "very little profile development has taken place. Because of the very sandy character of the parent material and the practical absence of silt and clay, no well-developed podzolic texture profile is possible. Slight decomposition of feldspar seems to have taken place, and a small amount of organic matter has accumulated in the upper 2 or 3 inches. The true Podzol profile is faintly developed in some of these sands, and it is



probable that, before the region was settled by white men, most if not all the areas had such a profile, but because of the smooth relief on which these sandy soils lie and the absence of boulders they were cleared and plowed at an early date. Thus the Podzol profile was destroyed."

A hypothetical "typical" soil profile, characteristic of most of the grassland and forested areas on the plains, and disregarding surface organic accumulations, may be described as follows:

- A. Thickness—8 inches; color—dark brown to very dark grayish brown, blackened when wet; texture—coarse sand; structure—single-grained, sometimes partially granular; consistency—loose. Lower limit well marked.
- B. Thickness—22 inches; color—yellowish brown or brownish yellow, often with a reddish cast; texture—coarse sand, sometimes loamy; structure—single-grained, sometimes partially granular to slightly cloddy; consistency—loose to friable, rarely firm.
- C. Thickness—variable, to underlying clay, till, or bed rock; color—variable, mixtures of gray, brown, yellow and red; texture—coarse sand, occasionally finer or coarser with gravel admixture; structure—single-grained; consistency—loose. Stratification present owing to nature of original deposition.

The sharp, even lower limit of the A horizon at an approximate depth of 8 inches was thought at first to represent the lower limit of disturbance by plowing; but its presence in areas which would seem to have escaped cultivation (such as ancient fence lines and more or less isolated projections of the terrace on the western margin), the general absence of lighter colored material in this horizon, and the occasional greater depth to which its lower limit reaches, seem to negate such a hypothesis. While the disturbances which cultivation produces in a normal profile are recognized, it was felt that the evidence just given seemed to demand the assumption that large areas of this terrace had carried a persistent grassland cover prior to white colonization. This type of cover could easily account for the blackened character of the A horizon, and it is not probable that this character would be lost in the relatively short period of time

since settlement.<sup>4</sup> The sharp lower limit is not easy to explain, but possibly coincides with the lower limit of the greatest average concentration of grass roots. Following grass fires, carbon particles might also be carried to an approximately uniform depth by percolating water.

Nearly all of the profiles examined which do not agree with the preceding description may be referred to truncated or buried variations of this type. Those in forest or scrub often show incipient podzolization under a relatively thin humus layer. This layer seems to approach the root or leaf duff of ROMELL and HEIBERG (49), although it is not well developed in many cases. In some extremely local areas no profile differentiation could be recognized, but no definite suggestions have been made to account for this fact.

It would be expected that these soils are low in soluble nutrients. A summary of data for Merrimac coarse sand and related types from the papers of MORGAN (41) and LUNT (36) shows that they are lower in total nitrogen and total phosphorus than any other soil in Connecticut, and are near the lower limits in total calcium and magnesium. Total potassium approximates the average of all Connecticut soils, which are all low in available potassium. There is reason for believing that the sand plains soils are lower than the average in available potassium. Replaceable calcium is undoubtedly low, as indicated by the generally high acidity. This condition also suggests the presence of both soluble aluminum and manganese. The actual occurrence of these elements in soluble forms, which are apparently toxic to many plants, has been shown in tests by MORGAN.<sup>5</sup> Available phosphorus apparently occurs in much greater amount in the sand plains soils than in many other Connecticut soils.

## Vegetation

### REGIONAL CLIMAX

The climatic climax of southern Connecticut is probably a mixed mesophytic deciduous forest, with or without an admixture of hemlock. NICHOLS (42) states that "the ultimate or climax formation at-

<sup>4</sup> These views of the writer have been substantiated by Mr. M. F. MORGAN in personal communication.

<sup>5</sup> Personal communication.

tained in the region under surveillance [Connecticut] . . . is a forest composed largely of deciduous trees and hemlock." He names approximately fourteen species of trees as important components, but goes on to state that "the forest is by no means uniform in structure throughout the state. Most widely disseminated and of greatest economic importance is the "sprout hardwood" type which represents the usual climax formation over fully five-sixths of the state. This type of forest attains its highest development in the central lowland and along the coast, where it is dominated by chestnut, oaks and red maple." Chestnut, of course, has since been eliminated as a tree. SHANTZ and ZON (51) mapped southern Connecticut in their chestnut-chestnut oak-yellow poplar forest. LUTZ (38) said that "there seems to be little question that hemlock-hardwood is the climatic climax association on the upland soils of southern New England." BROMLEY (9) considered that the "typical mixed mesophytic forest" composed of oak, chestnut, hickory, sugar maple, beech, tulip poplar, and a varying admixture of eastern hemlock represents the climatic climax in his general "oak region" of southern Connecticut, Rhode Island, and Massachusetts. NICHOLS (45) drew the boundary between his "hemlock-white pine-northern hardwood forest" and deciduous forest so as to include southern Connecticut in the last named.

No climax forests of the kind here suggested now exist on the sand plains under consideration, and there is little evidence to indicate that they have been present in the past. Species commonly associated with mesophytic climax forests are absent, or rare and of local occurrence, on these level terraces. In the opinion of the writer, there is insufficient evidence for assuming that, even barring disturbance, forests of this type would develop to any great extent in the future. The podzolization inherent in the maturation of the sandy soil profile under present climatic conditions will accentuate rather than diminish many of the edaphic factors inimical to such forests. The most stable and relatively permanent types of vegetation now found on the sand plains may therefore be considered as climaxes limited by fire, man, or soil, alone or in combination. Whether we call them climaxes, qualifying the term by the descriptive adjective of the differentiating factor, such as edaphic, physio-

graphic, biotic, or fire, as does TANSLEY (53), or consider that they fall under some one of the proclimaxes of CLEMENTS (13), would seem immaterial, except that the proclimax must be "gradually replaceable by the latter [the climax] when the control of climate is not inhibited by disturbance." The difficulty involved in calling them all proclimaxes hinges upon the possibility of designating as disturbances such relatively permanent characters as coarse sandy soil and increasingly inhibitory influences connected with podzolization.

#### COMMUNITY RECOGNITION AND CLASSIFICATION

The actual delimitation and classification of all the plant communities found in the areas of study involve certain difficulties. Since a very detailed causal analysis was desired, the recognition of associations and consociations (associes and consocieties of CLEMENTS) along broad general lines was inadequate for careful correlation. Likewise the actual areal units are in many cases too small to allow such recognition, forming, as they often do, a mixture or mosaic of various types. Perhaps some communities might be recognized as fragmentary associations. In general the writer does not wish to assign some of his units to places in any of the various systems of classification of vegetational units, such as those of NICHOLS (44), BRAUN-BLANQUET (6), or CLEMENTS (13).

The arrangement of the units in one psammosere, following NICHOLS' treatment (43), is likewise inadequate, since it will be shown later that the area includes three or more subseres defined on the essential characters of the initial habitat.

It is thought that the method of classification finally adopted will allow the reader to visualize and understand the essential ecological characters, the causal relations, and the successional sequence of the various plant communities without serious difficulty. It is based upon habitat, physiognomy, floristic composition, and succession with varying emphasis.

The three or four physiognomic types of vegetation involved have already been suggested, and their general distribution in the area of study can be seen in figures 1-3. Light-colored areas are either barrens or cultivated fields. The latter are not considered, and can easily be recognized by their more uniform tone and the lack

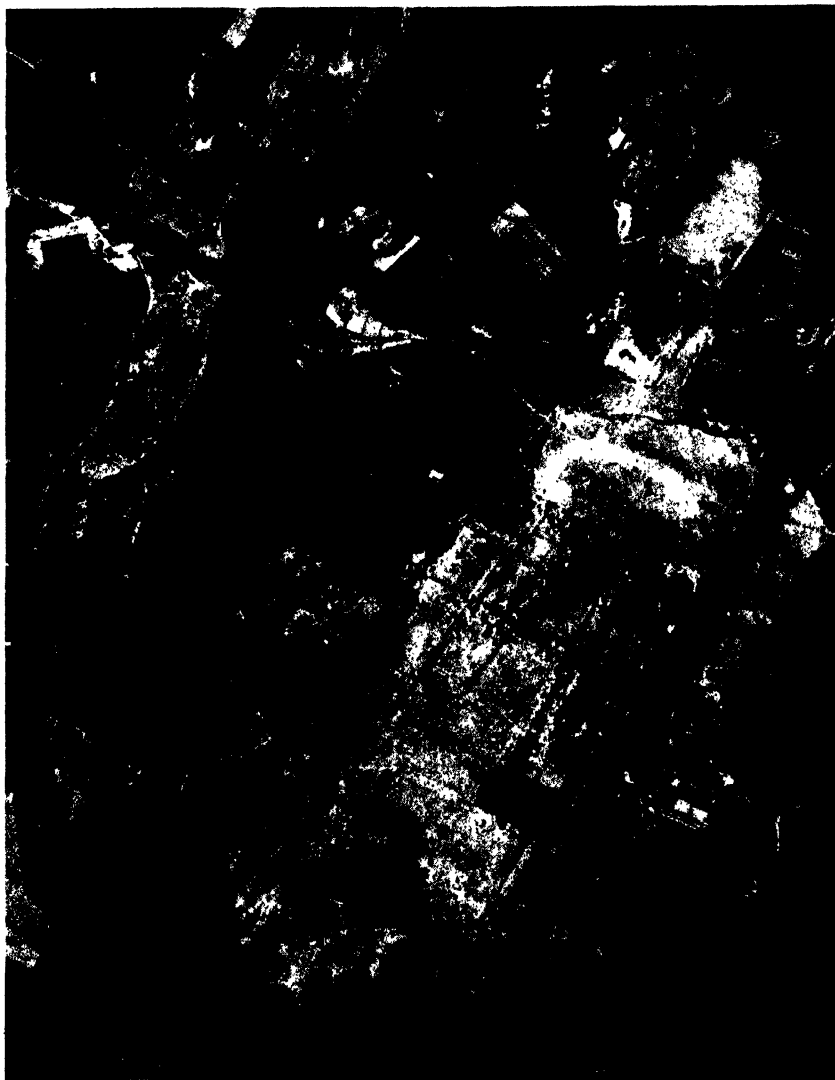


FIG. 1.—Figs. 1 to 3 are aerial photographs representing 1 mile in width, taken May 1934, of portions of townships of Wallingford and North Haven, showing most of the areas of study. Quinnipiac River is on the left. Eastern and western margins of terrace shown approximately by the black lines. Light tones indicate barrens, especially the *Trichostema-Andropogon* community, or cultivated fields; medium tones, the *Andropogon-Cladonia* association; dark tones, dominant woody vegetation; very dark tones, water table above the surface. Fig. 1 shows southern end of borough of Wallingford in upper right corner. Note extensive development of barrens, *Andropogon-Cladonia* association, and fence row communities. Reproduction by courtesy of Fairchild Aerial Surveys, Inc.

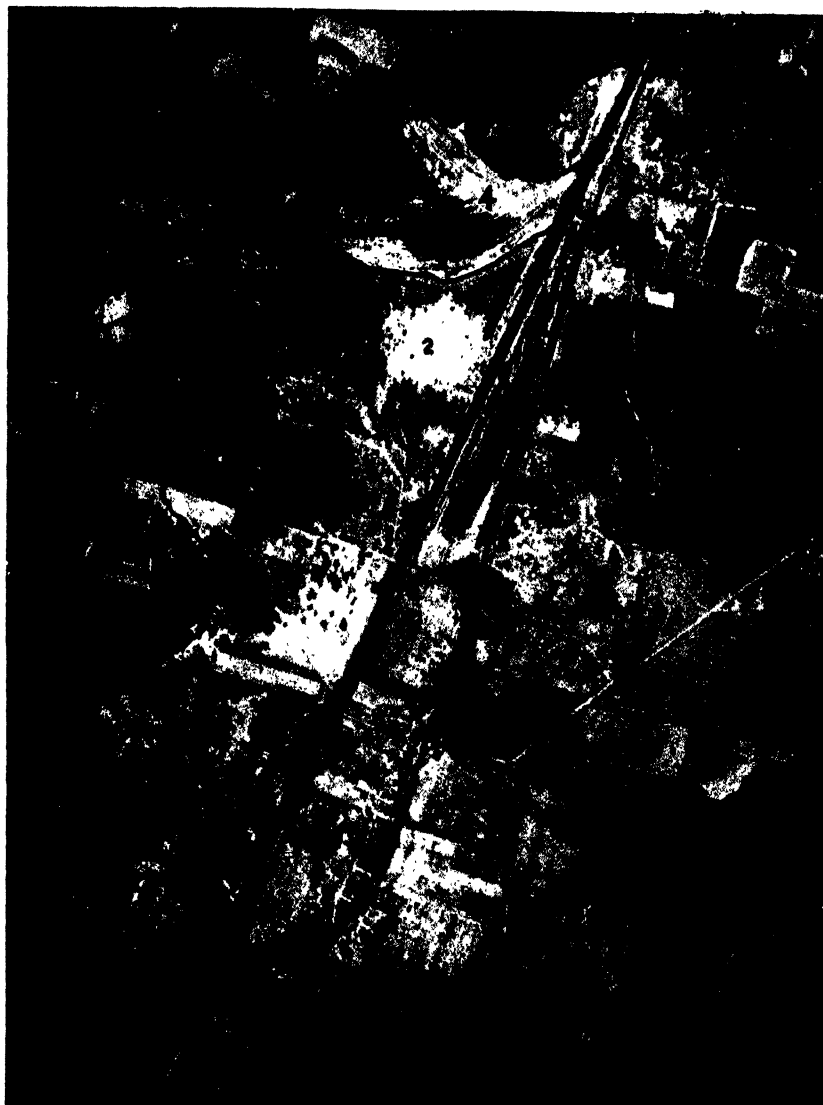


FIG. 2.—The stream near the upper margin, also visible at bottom of fig. 1, is the boundary line between North Haven and Wallingford. Two pitch pine stands, and several stands of the *Andropogon-Cladonia* association may be seen. Reproduction by courtesy of Fairchild Aerial Surveys, Inc.



FIG. 3.—Continuation of the area south of the portion shown in fig. 2. Portion of village of North Haven at lower left corner. Barren areas on left were among those studied by BRITTON (8). Reproduction by courtesy of Fairchild Aerial Surveys, Inc.

of darker patches which are found in the barrens and which indicate trees or grass. The term barrens is here used to designate all areas in which the surface soil is largely devoid of living vegetation or a cover of dead plant parts, so that bare sand is much in evidence, especially in winter. In some cases this includes woody vegetation with a nearly complete canopy. Areas of medium tone are grasslands in which the surface of the soil is almost completely covered by *Andropogon scoparius*, a mixture of lichens, mainly *Cladonia* spp., and mosses, as well as other herbaceous or semi-woody plants. These areas also show sporadic occurrence of trees and shrubs; the actual condition often resembles parkland or savanna. Areas of darker tone indicate dominant woody vegetation, either scrub or forest. The darkest tone is given by woodlands or forests of pitch pine.

It was suggested that most of the soil profiles could be referred to the typical profile which was described, or to truncated or buried variations of this type. This fact forms a basis for recognizing three important initial habitats. The nature of these has considerable influence upon the time and manner of origin, and upon the floristic composition and ecological character of the various successional stages. The most important initial habitat (type 1) is that of the "typical" profile already described. The next most important (type 2) is one in which the A horizon has been entirely removed, with varying amounts of the B horizon, primarily by wind erosion, aided in some cases by surface washing. The third (type 3) is that in which deposition of sand by wind has brought about the formation of ridges and hummocks of varying depth overlying the typical profile. The essential relations of these habitats are shown in figure 4, and data for some of their important soil characters are given in a later section.

Barrens are found on all three types of habitat, and communities of this nature are practically the only ones found on type 2. Type 1 is covered primarily by grassland, scrub, or forest, but occasionally is barren, while type 3 is usually barren but sometimes shows sufficient ground cover to justify its removal from this category.

Annual species are important in the earliest stages of succession on the barren areas. Three species, *Hypericum gentianoides*, *Tricho-*



*stema dichotomum*, and *Polygonella articulata*, may be considered as indicators of initial habitats 1, 2, and 3 respectively. Although each species is not an exclusive<sup>6</sup> in the designated habitat or habitats, it does attain its highest values of abundance, cover, and frequency

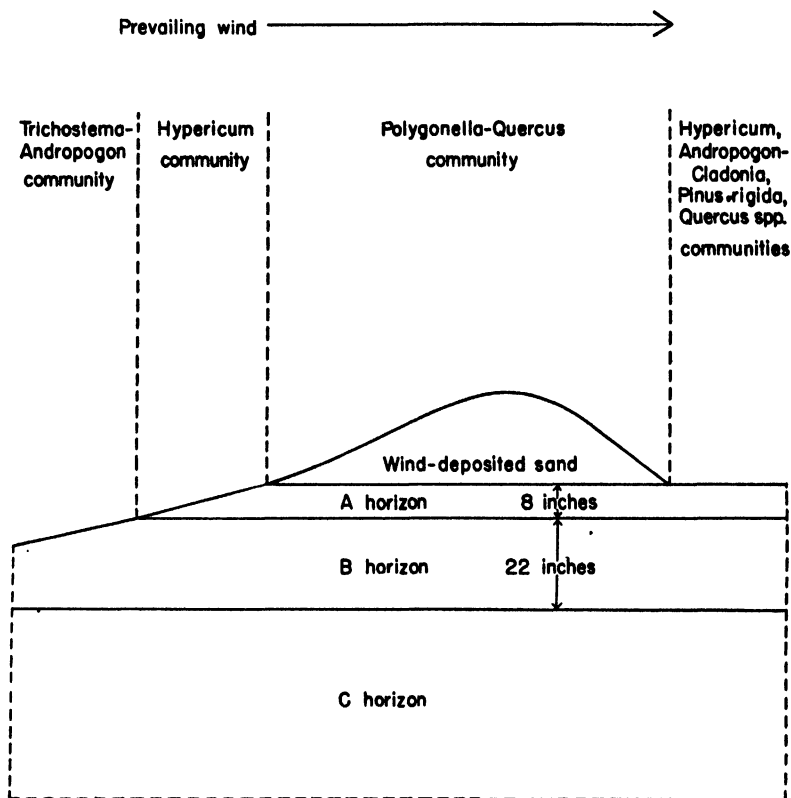


FIG. 4.—Cross sectional diagram (not drawn to scale) illustrating major edaphic habitats on sand plains, and correlated plant communities.

within areas of that type, and shows a high value for presence. It is logical, therefore, to utilize the species name in designating the community. *Trichostema* is also one of the most abundant and frequent species on railway embankments and roadbeds, and on completely unweathered sand exposed in gravel pit excavations.

<sup>6</sup> This term and most of the other "phytosociological" terms are used in the sense of BRAUN-BLANQUET (6).

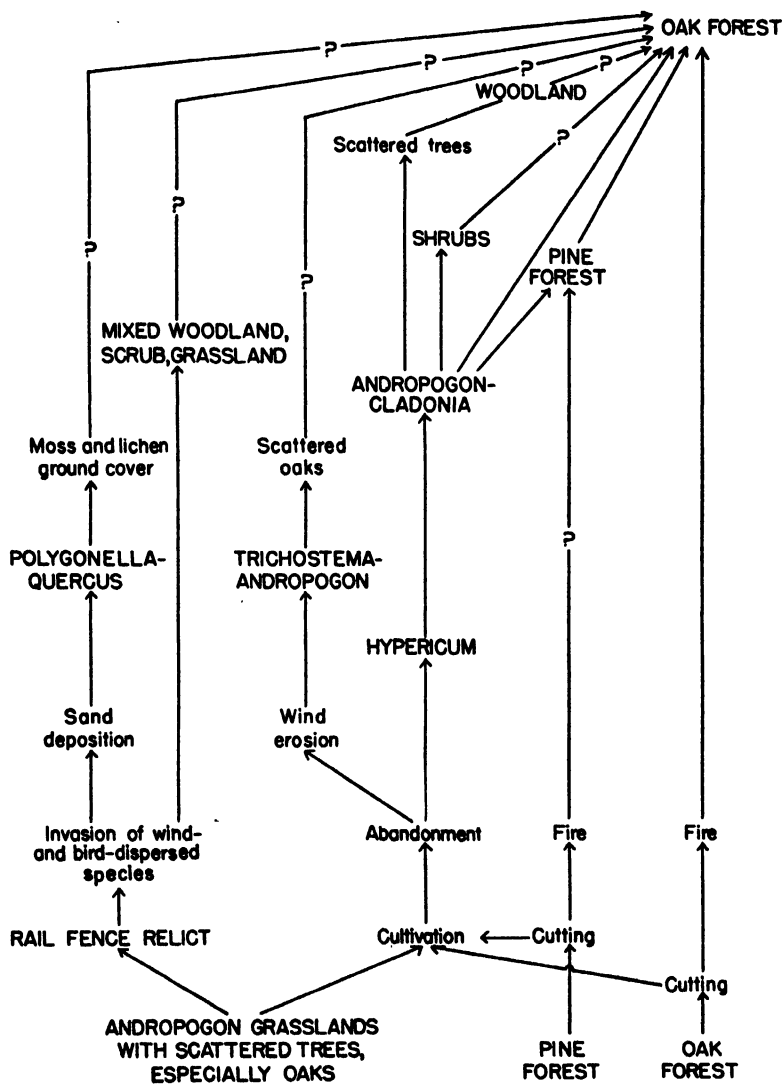


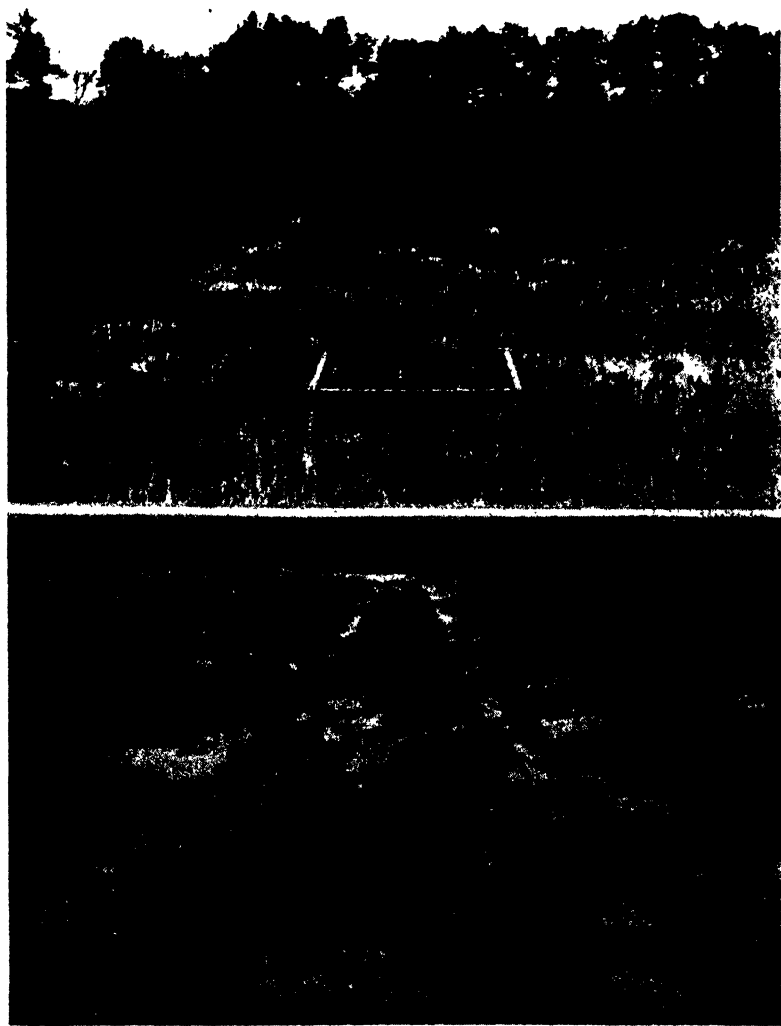
FIG. 5.—Diagram of probable past and present successional relations of sand plains communities.

## HYPERICUM COMMUNITY

The *Hypericum* community (figs. 6, 9) is characteristically found on exposed lower windward slopes of wind-formed ridges and hummocks, in the region of outcrop of the A horizon (shown in figure 4); therefore it often appears as a long narrow strip. Further successional development is apparently slow, and only a few other species, especially *Andropogon*, are found, although the habitat is open and much more favorable in its edaphic relations than is one with A horizon entirely lacking. It is interesting that the only pitch pine within the barrens communities was found on such a slope. The *Hypericum* community may also be well developed in local areas within the grassland community with intact A horizon (shown in figure 6), and may represent the first successional stage here. It is invaded by *Andropogon* and lichens, and later probably gives way completely to the grassland community, although the dominant species may persist as a relatively unimportant constituent within the grassland. Since *Hypericum gentianoides* is an annual, areas dominated by it are usually without a protective vegetative cover from the last of autumn until late spring, for it is tardy in germination and growth. This is brought out in the data for the same quadrat in 1a and 1b in table 1. In spite of late germination, however, many individuals are in flower by the middle of July. The general relation of *Hypericum* and *Trichostema* is illustrated in quadrat 5, showing a relative decrease of the first and an increase of the second, correlated with the fact that most of the A horizon was missing in this quadrat. Quadrat 6 is shown in figure 6.

Within the *Hypericum* community the species usually fails to attain the size which it often exhibits as a ruderal in fine sandy soils. Thus among the thousands of specimens counted in the six quadrats, the maximum height measured was 17 cm., and the average height of the largest plants was not more than 10 cm. Many of them were but 1-2 cm. tall and consisted of an unbranched shoot with a solitary flower or fruit. Root systems seldom exceeded 6 inches in depth.

A relatively high frequency of the annual *Stenophyllus* is indicated by the data given, and it is a more or less constant though unimportant member of the community. The situation changes in a cer-



FIGS. 6, 7.—Fig. 6 (above), *Hypericum* community in foreground, with quadrat 6 (table 1). This community is being invaded by the *Andropogon-Cladonia* association in immediate background. Black oaks in an old fence line visible in distance. September 19, 1935. Fig. 7 (below), *Stenophyllus capillaris* and *Hypericum gentianoides* in and near tracks of little-travelled road in *Andropogon-Cladonia* association. August 20, 1935.

tain variant of this community often found in tracks and along the edges of little-travelled roads and trails within the grassland (fig. 7). Here *Stenophyllus* becomes dominant, and the abundance of *Hypericum* decreases, although the average size of individuals is somewhat greater than in the typical community. Although no careful study was made, the root-top ratio in these two annuals seems lower than that of *Trichostema*. Their greatest abundance upon soils with an

TABLE 1

ABUNDANCE BY METER QUADRATS OF SPECIES IN THE HYPERICUM COMMUNITY

SPECIES	QUADRAT						
	1a	1b	2	3	4	5	6
<i>Polytrichum piliferum</i> ...	.....	.....	0*	0*	.....	.....	.....
<i>Andropogon scoparius</i> ...	.....	.....	.....	2	.....	.....	2
<i>Panicum depauperatum</i> ...	.....	.....	.....	.....	1	1	.....
<i>Stenophyllus capillaris</i> ...	3	2	5	.....	7	2	.....
<i>Hypericum gentianoides</i> ...	2,961	3,218	1,042	610	428	145	4,946
<i>Trichostema dichotomum</i>	58	43	16	10	29	80	.....

\* 0 Limited occurrence but impossible to count.

Dates of counting: 1a, July 17; 1b, August 21; 2, 3, 4, 5, August 20; 6, September 19. The grass plants were seedlings.

A horizon may be correlated with that fact, for such soils have a higher water-holding capacity and would be expected to be higher in available nutrients, especially nitrogen.

#### TRICHOSTEMA-ANDROPOGON COMMUNITY

This is the most important community in the barrens found on soils with A horizon lacking. The individuals of the two species vary in frequency and abundance from place to place, often occurring more or less sporadically, but also in definite patches or rows, as shown in figures 9 and 10. These concentrations seem to arise through the accumulation of fruits and seeds in footprints and in wheeltracks of wagons and automobiles, through wind action. The disseminules are almost immediately covered by drifting sand or by surface washing to a depth of 0.5-2 inches, for tracks are soon obliterated. The importance of immediate burial in the barren areas cannot be overemphasized, especially when dealing with a

species such as *Andropogon* with fruits well adapted for wind dispersal. If they were not quickly covered, most of them would be carried out of the barren areas. This is not so true of seeds of the



FIGS. 8, 9.—Fig. 8 (above), stand 1a and 1b (table 2) of *Trichostema-Andropogon* community. Oaks and bare sand of *Polygonella-Quercus* community visible in background. July 23, 1935. Fig. 9 (below), *Trichostema dichotomum* in automobile tracks, and scattered clumps of *Andropogon scoparius*. *Hypericum* community at upper left with *Polygonella-Quercus* community just above it. *Andropogon-Cladonia* association and scattered oaks at upper right. September 19, 1935.

annual plants, which are so small that many of them seem to be carried downward to a depth of 0.25–0.5 inch in the loose surface sand by percolating rain water.

In addition to the importance of the tracks in catching fruits and seeds of these and certain other species, they also induce better conditions for cecis. The surface inch or so of these barren areas is always blackened with organic and inorganic dust. Therefore, in spite of the fact that the immediate surface usually shows a desert pavement of very coarse sand and small gravel particles and pebbles, caused by deflation, the surface inch is higher in water-holding capacity, loss on ignition, and probably in available nutrients than are the lower layers. The mechanical effect of man and machine through tracking is to carry this surface layer downward *en masse*, resulting in an improvement in the general edaphic relations of the lower layers, often to a depth of 4 to 5 inches. Since the deposits which accumulate in the tracks are likewise rich in organic and inorganic dust, the total effect is a gradual though sporadic improvement of the habitat. A certain amount of seed burial and soil mixing is also brought about by the activity of moles. These effects of burial and improvement must account in large measure for the great increase in abundance and frequency of *Andropogon* on most of the barren areas during the past 35 years which is plainly visible in photographic records.<sup>7</sup> Thus it seems clear that eventually this type of barren area may be reclaimed by a more or less continuous grassland cover. As yet, however, there is little indication that such grasslands will soon resemble those now in existence in their details, since lichens are not invading the bare sand between the clumps of grass as they do in the grassland communities developed on the profile with A horizon intact. Since observation shows that actual abundance and frequency of living grass clumps may be as great in one community as in the other, failure of lichen establishment probably does not result entirely from removal of propagules from the barren areas by wind sweep. The suggestion is advanced that many, if not most, of the lichens available for colonization are species which require a certain amount of organic material in the substratum for successful establishment. This material is mainly lacking in the truncated soil profile. *Cladonia cristatella* f. *abbreviata* is sometimes found on dead leaves within the clumps of grass. This species is notorious for its "humus requirement."

<sup>7</sup> Personal communication from G. E. NICHOLS.

AUTECOLOGY OF *TRICHOSTEMA*.—Comparison of the permanent quadrat data for abundance in stand 1a and 1b in table 2 (this stand is shown in figure 8) indicates the death of many individuals of *Trichostema* in the eight weeks' interval. That habitat conditions are near the extreme limits of toleration by this species is also shown in the fact that only 374 of the 567 individuals still alive on August 21 showed any indication of flowering. Most of the others had but

TABLE 2

METER QUADRAT DATA FOR *TRICHOSTEMA*-*ANDROPOGON* COMMUNITY.  
QUADRATS SPACED 50 FEET APART ON ONE OR MORE LINES

SPECIES	1a			1b			2		
	20 QUADRATS						25 QUADRATS		
	A*	F%	C%	A	F%	C%	A	F%	C%
Andropogon scoparius (ma- ture).....	21	80	8	21	80	8	10	24	3
Andropogon scoparius (seed- ling).....	14	30	.....	16	30	.....	50	60	.....
Panicum depauperatum (ma- ture).....	4	15	1	4	15	1	8	20	2
Panicum depauperatum (seed- ling).....	22	25	.....	24	30	.....	61	44	.....
Panicum spp. (mature).....	1	5	.....	1	5	.....	.....	.....	.....
Panicum spp. (seedling).....	7	20	.....	8	20	.....	4	16	.....
Stenophyllus capillaris.....	2	5	.....	4	10	.....	8	4	.....
Polygonella articulata.....	.....	.....	.....	.....	.....	.....	135	88	.....
Hypericum gentianoides.....	4	10	.....	8	10	.....	.....	.....	.....
Asclepias amplexicaulis.....	.....	.....	.....	.....	.....	.....	1	4	.....
Trichostema dichotomum.....	788	100	.....	567	100	.....	2,930	100	.....

\* A, abundance in all quadrats; F, frequency %; C, cover %. Dates of counting: 1a, June 29; 1b, August 21; 2, July 17.

one or two pairs of leaves in addition to the cotyledons, and some showed only one cotyledon. The height range of 0.3 to 8 cm. was in marked contrast to that of 25 to 50 cm. which the species often attains in favorable habitats.

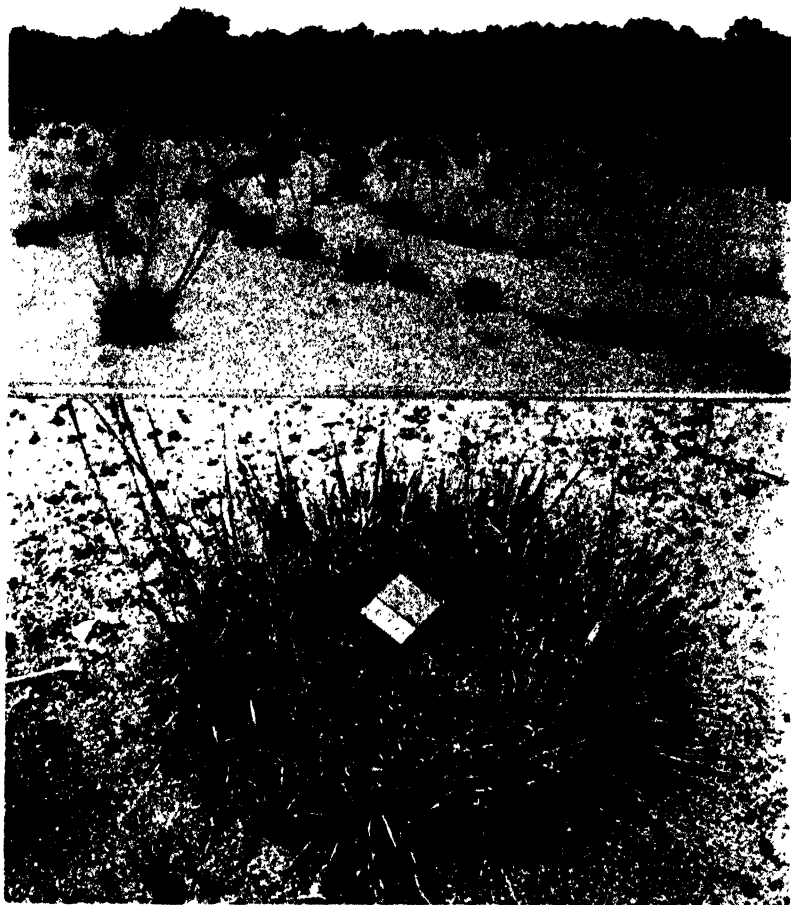
*Trichostema* shows a longer period of activity than does *Hypericum*. On April 27, 1933, a close examination of the surface of the sand showed many cotyledons of the first species already in view. Roots had attained a length of 4 to 5 cm. No further growth above ground was evident until the first week in June. May was a month of severe



drought; nevertheless the root systems were being extended. Mature individuals 6 to 8 cm. in height often have root systems extending to depths of 20 to 25 cm., with a lateral spread of the same magnitude. *Trichostema* rarely flowers before the first week in August, when the larger individuals produce flowers and fruits in abundance.

AUTECOLOGY OF ANDROPOGON.—*A. scoparius* is the most important species in the sand plains vegetation as a whole, occurring as a more or less important constituent in all of the barrens communities and as a dominant in the *Andropogon-Cladonia* association. Within the barrens effective burial of fruits of this species and consequent ecesis seem to occur mainly in the manner just described. Seedlings once established seem to have a strong hold on life, although their increase in size above ground is very slow. Probably few clumps produce flower stalks before they reach an age of 5 or more years, in spite of absence of competition. WEAVER and FITZPATRICK (57) state that this species may flower and fruit the first year in the absence of competition, and within 2 to 3 or more years in the prairie. Many of the plants on the sand plains, however, grow to form clumps which are 2 to 3 feet or more in diameter in contrast to the maximum size of 1.5 by 2 feet, or the more usual diameter of 6 to 8 inches recorded in the prairie, by WEAVER and FITZPATRICK. It undoubtedly takes many years for them to reach such a size. A characteristic feature of such clumps is death of the central and growth of the peripheral portion to produce "fairy-ring" circles. An early stage in this development is seen in figure 11. Since the desert pavement is not complete in barrens with A horizon lacking, deflation of the general surface is still going on slowly. When a clump of *Andropogon* has reached a diameter of 2 to 3 inches, it not only tends to prevent removal of sand from underneath it, but also catches wind-blown material. Consequently, with increased growth of the clump, continued accumulation of sand within it, and continued deflation outside, the relative level of the clump becomes higher and solid cushions of sand and grass are formed up to 6 inches in height. In larger fairy-rings, the surface of the central portion may consist entirely of wind-blown sand into which one must dig to a depth of 1 inch or more to uncover the dead leaves and culms of

earlier growth. That the death of the central portion is not caused entirely by sand burial is indicated by the fact that WEAVER and



FIGS. 10, 11.—Fig. 10 (above), *Trichostema-Andropogon* community showing development of *Andropogon* clumps in parallel rows, resulting from accumulation of fruits in wheel tracks. *Quercus* spp. association growing on western margin of terrace in background. June 29, 1935. Fig. 11 (below), fairy-ring of *Andropogon scoparius*. Average diameter of clump is 16 inches and average basal level is 2 inches above that of surrounding sand. Desert pavement well shown. August 20, 1935.

FITZPATRICK recorded a similar deterioration of clumps in the prairie region, where no burial occurs.

Above-ground parts of a plant of *Andropogon* are not indicative of the total bulk of the individual. The root-top ratio is very high. Thus an isolated plant with an estimated age of 4 years had approximately one hundred leaves 3 to 5 inches in length, and a number of shorter ones. Its basal area was approximately 2 square inches, with a top spread 5 inches in diameter. The root system consisted of seventy main fibrous roots, which branched repeatedly. Tips of some of these roots were found at depths of 33 inches directly beneath the plant, but most of them extended horizontally within the surface 15 inches of sand, greatest concentration occurring at the 6 to 10 inch level. The straight-line distance from the farthest tips of many of these laterals to the base of the plant was often 4 feet. Many secondary roots branching off from these laterals at intervals of 0.5–1 inch extended horizontally at right angles for distances of 6 to 10 inches, while others extended directly upward to within 2 inches of the surface. No roots were found within the surface 1.5 inches of sand. Thus the roots of the plant described were ramified throughout more than 50 cubic feet of soil. Probably the actual volume of soil occupied by the roots of any one clump does not exceed this amount to any considerable degree, no matter what the size or age of the clump. In any case, the maximum depth recorded for *Andropogon* roots in any one of numerous excavations was 50 inches, and the horizontal extent was nowhere more than 4 feet from the periphery of the clump. In all cases, most of the roots were concentrated in the surface foot of sand, even in the *Andropogon-Cladonia* association where there is some competition between individuals. This general root habit may be contrasted with that of the same species in the prairie region under conditions of severe competition, where it usually reaches depths of 5 feet but has a much smaller horizontal spread.

ROOT SYSTEM OF *Panicum depauperatum*.—This species is the next most important grass in the sand plains, and is nearly always associated with *Andropogon scoparius*. It also exhibits a high root-top ratio. A mature individual with a basal area 3 inches in diameter, an average height of 4 inches, a top spread 1 foot in diameter, 96 culms, and 208 green leaves, possessed 261 main roots at a distance of 1 inch from the base of the clump. The system of spreading is

somewhat different from that of *Andropogon*; the roots are considerably finer, and are more easily broken. A much greater number seem to extend vertically downward. In the plant described these reached depths of 24 to 33 inches. Other roots extended horizontally for 1 foot or so at a depth of 2 to 6 inches and then turned directly downward to reach depths of 10 to 24 inches. Still others continued their horizontal spread 18 to 24 inches. The maximum length recorded for a main root was 38 inches. The main roots give rise to innumerable branches, which reach varying lengths, but are so easily broken by falling sand that excavation is almost impossible. As many as 16 branches per inch of main root were recorded.

INVASION OF OTHER SPECIES.—Small mounds of sand 2 to 3 inches in height also tend to accumulate around the larger bunches of *Panicum depauperatum*, although this species does not form fairy-rings as does *Andropogon*. Disseminules of these two grasses and those of other plants are buried in these peripheral accumulations of sand, thus obtaining favorable conditions for germination. The seedlings recorded in table 2 mainly have such an origin. Most of them fail to survive, and this failure may be caused by further sand shift, or by mechanical uprooting through the sweeping action of the long dead culms of *Andropogon* whirled in a circular direction about each clump during high winds. Some do survive, however, and this method of burial, plus that in tracks, probably accounts in large part for the occasional occurrence of a number of species of other perennial plants in the *Trichostema-Andropogon* community. Prominent among these are *Helianthemum majus*, *Baptisia tinctoria*, *Lespedeza capitata*, *Asclepias amplexicaulis*, and *A. syriaca*, all of which develop deep and widely spreading root systems. The tap root of a vigorous plant of *Baptisia* 3 feet high was found to reach a depth of 55 inches, where it broke up into a cluster of small roots. It gave off a number of thick horizontal and descending laterals, some of which reached a like depth, or extended horizontally as far as 6 feet from the base of the plant. The total length of the longest lateral, and of the tap root from the origin of the lateral to the surface of the soil, was 9 feet.

AUTECOLOGY AND IMPORTANCE OF *QUERCUS VELUTINA* IN THE COMMUNITY.—Two species of oaks invade the *Trichostema-Andro-*

*pogon* community. Of these, *Quercus velutina* is much more important than the dwarf *Q. ilicifolia*. The origin, development, and implications of the first named species have been carefully studied. BRITTON (8) was undoubtedly correct in attributing the presence of black oaks in certain of the barren areas mainly to the burial of acorns by squirrels. Very few oaks under 10 years of age are found within the *Trichostema-Andropogon* community, the *Hypericum* community, or even the *Andropogon-Cladonia* association, for which such an origin cannot be demonstrated easily. The same is true to a lesser degree in communities with a substratum of wind-borne sand. Without dispersal by squirrels the acorns would not enter many of the areas, and even though they did, no establishment would occur without burial.

Acorns in squirrel caches are usually buried to a depth of 2 to 4 inches, each cache being from 1 to 3 feet in length and 2 to 3 inches wide. The range in number of acorns found in many caches was twenty-three to eighty-four and the average about forty. The fate of these acorns varies. Thus one cache of fifty-eight acorns (fig. 12), apparently buried in the fall of 1933 to a depth of 4 inches, showed but one oak seedling above ground in the summer of 1935. The acorn had germinated in the spring of 1934. Twenty-eight of the acorns had failed to germinate. The other twenty-nine acorns had also germinated in the spring of 1934 and the embryonic plants had developed vigorous tap roots 8 to 14 inches long. All were still alive when the cache was excavated in 1935 but not one had sent a shoot above ground. The shoots had grown upward about 2 inches, when many of the terminal growing points died, and branches arose from the cotyledonary buds or other lateral growing points. These also failed to reach a level higher than 1.5 inches below the surface. At this level both terminal and lateral growing points extended in a horizontal or downward direction. Possible explanations for such erratic behavior might be that the acorns were buried too deeply, or that the high surface soil temperatures of the open sand or its usual dry condition might be responsible. In a cache which was a year older, two out of twenty-seven acorns had produced seedlings, two had failed to germinate, and the other twenty-three had performed in a manner similar to that described, some of which were still alive

2½ years after germination. Another cache, 4 years old, showed ten seedlings, eleven ungerminated acorns, and two with erratic be-



FIGS. 12, 13.—Fig. 12 (above), partial excavation of black oak seedlings in squirrel cache. Top of ruler is at surface level. July 3, 1935. Fig. 13 (below), two adjoining groups of oaks with origin from squirrel caches in *Trichostema-Andropogon* community. Group to right is 9 years old. *Quercus* spp. association in background, growing on western margin of terrace. July 23, 1935.

havior. The largest oak with unquestionable origin from a squirrel cache was an isolated specimen 54 inches tall, apparently 12 years

old. Forty-two rotting acorns were recovered near its base. Nine of them showed evidences of germination, but none had developed shoots above the surface.

Immediate success is not assured for those oak seedlings which get above ground. Defoliation by insects, death of the terminal growing point through various causes, and other injurious influences bring about a high mortality, and those which survive are kept in an

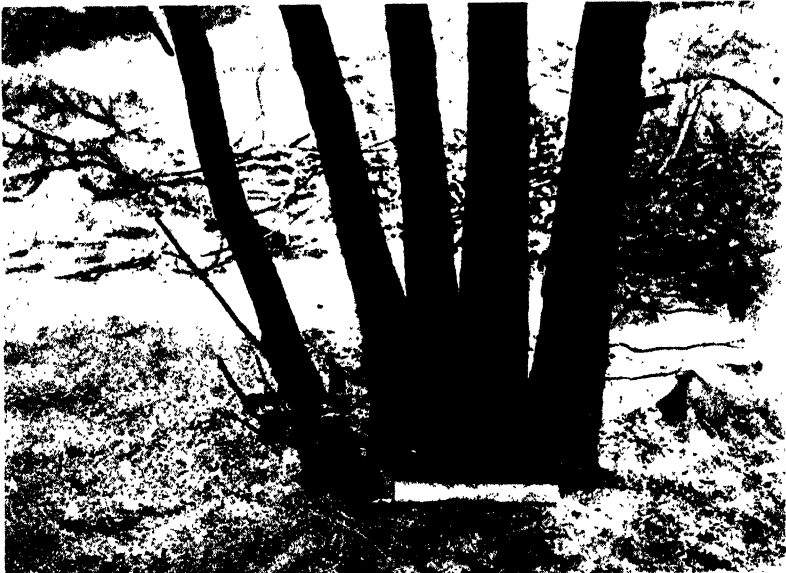


FIG. 14.—Five black oaks, with separate bases and root systems, showing origin from squirrel cache in *Trichostema-Andropogon* community. August 31, 1935.

aerially suppressed condition for 5 or 6 years. During this time, however, the root systems are growing vigorously. Plants which reach ages of 10 years seem relatively certain of survival, although they may not reach or exceed 2 feet in height. By this time all but the most vigorous specimens have been eliminated. These usually range in number from one to three per cache, and from this period onward undergo a very rapid increase in growth rate, so that trees may be 50 feet tall at an age of 50 years. Many trees, however, never attain such a rate. Usually one or two trees in each cache gain the ascendancy. One group of four, of which the tallest was 47 feet, showed

trunk diameters breast high of 13, 12.5, 7.5, and 5.5 inches, with ages at the same height of 38 years. The bases were grafted together through pressure caused by diameter increase, but it seems very probable that their origin was in a squirrel cache. Fully half of the largest oaks in grassland and barrens show this type of grouping. This might also arise through basal sprouting after injury, but all the evidence seems to indicate that it is probably caused more often by common origin in a squirrel cache. This is evident for the trees shown in figures 14 and 21.

The rapid and extensive growth of the root systems of black oaks has already been mentioned. Scattered data to support this statement were obtained in soil pit excavations, as well as in direct excavation of seedlings. Seedlings 2 or 3 years old usually showed a well developed tap root about 2 feet long. This gave rise to two or three large laterals in the upper foot, which extended more or less horizontally 12 to 30 inches. The tap root and these laterals also bore numerous small short roots.

The most careful excavations were those which uncovered the roots of the eight small oaks shown on the right hand end of the row in figure 13. These trees had grown from acorns in a cache about 1 foot long. Forty acorns were recovered at a depth of 3 inches. The trees were 9 years old, and the total length of their main stems varied from 12 to 26 inches. The soil showed a truncated profile with the old A horizon entirely missing, and the bottom of the B horizon at a depth of 17 to 18 inches. The largest tree carried 331 leaves with a total area of 366 square inches on twenty-two, thirty-one, and six primary, secondary, and tertiary branches respectively. The combined stem length was 198 inches. The 39 inch tap root broke up into many fine branches near its end, the deepest ending at 38 inches. Twenty-one important laterals arose from the tap root, fifteen of which started in the surface 7 inches of sand. These varied in length from 9 to 175 inches as measured, although breakage prevented complete measurement of some of them. Their combined length as measured was 984 inches. Making allowance for those which were broken, the total length of the important root branches of the first order thus was five times greater than the total stem length. But the lateral and tap roots also carried many fine branches which were not meas-



ured. Regardless of depth of origin, most of the laterals soon curved downward or upward to a 6-10 inch level, and extended in a straight horizontal direction at this depth. The next largest tree carried 167 leaves with a total area of 457 square inches on seventeen, sixteen, and one primary, secondary, and tertiary branches, with a combined stem length of 140 inches. This tree had a 49 inch tap root penetrating to 40 inches. Twenty-nine important laterals arose from it, with a combined length exceeding 1,186 inches. These varied in length from 6 to 167 inches, and sixteen of them had their origin from the upper 7 inches of the tap root.

These measurements indicate the great development of shallow lateral roots and a limited tap root development by the black oaks grown in the open. In some of the smaller trees the tap roots attained lengths of 130 inches, but did so by making an abrupt turn at a depth of 12 to 20 inches to pursue horizontal courses. Consequently most of the root system is found above the C horizon. This same type of root habit was indicated for forest-grown trees on normal profiles in soil pits among them. The yearly growth rate of the surface lateral roots is astounding. Laterals up to 18 feet long were found on a 12 year tree, while living roots up to 0.25 inch in diameter have been uncovered at a distance of 80 feet from the nearest oak, which was not older than 50 years.

LUNT (37) studied the distribution of moisture beneath such isolated trees on the sand plains. He found that only about 75 per cent of the precipitation reached the ground under large trees, and that after a dry period in summer, relative wetness values of the sand were lowest in the immediate vicinity of the tree base, and "increased in irregular zones with increased distance from the trunk, and with depth." In spite of this effect upon the soil moisture relation during dry periods, such trees serve as colonization centers through which other species invade the *Trichostema-Andropogon* as well as other communities in which such isolated trees occur. The effect of shade and shelter reduces evaporation of the surface soil moisture following rains, lowers the maximum soil temperatures, and hinders wind sweep. The actual conditions vary under each tree, depending upon the crown form, with high or low spreading

branches, and its effect upon sand accumulation and extent of shading.

Many of these trees in any type of barren area shelter large mats of *Polytrichum piliferum*, especially on the north sides (fig. 15). A crustose lichen, *Lecidea uliginosa*, is often associated with the moss. These mats with their rhizoids bind the loose sand to a depth of 1 to 2



FIG. 15.—Large black oak with a low fork, possibly representing two individuals with grafted bases, growing in *Hypericum* community island in *Andropogon-Cladonia* association. The picture, taken from the northeast, shows a mat of *Polytrichum piliferum*, *Lecidea uliginosa*, *Cladonia strepsilis*, and *C. cristatella* under the tree. Seedlings of black oak, gray birch, and *Andropogon scoparius* were growing on this mat. September 22, 1935.

inches, and collect wind-borne sand, dust, and plant disseminules. Fruticose lichens, such as *Cladonia mitis*, *C. caroliniana*, *C. uncialis*, and *Cetraria islandica*, soon come in on many of these mats (fig. 17) when the shade is not too dense, together with foliose and crustose forms such as *Baeomyces roseus*, *Cladonia cristatella*, *C. strepsilis*, *C. bacillaris*, *C. chlorophaea*, and *C. coniocraea*. These lichens may develop to such an extent that the moss is nearly eliminated. Other mosses, such as *Pholia nutans* and *Dicranella heteromalla*, may enter

these mats also, although they are more typically found under isolated trees in areas with a normal soil profile. Higher plants including oak seedlings also invade these mats and bring about their destruction. Some of these vascular plants may survive after the death of the sheltering oak, and form a permanent nucleus of woody vegetation. In other cases death of the oak results in the elimination of most of the species which developed in its shade.

The higher plants may also come in on open sand under the trees, even though no moss mat has developed. These species usually have either wind or bird-distributed fruits or seeds. Consequently the species population under large oaks varies considerably. Seedlings of *Betula populifolia*, *Prunus serotina*, *Quercus velutina*, *Andropogon scoparius*, *Panicum* spp., and *Baptisia tinctoria*, together with mature individuals of *Trichostema dichotomum* and *Asclepias amplexicaulis*, were found under one tree. Mosses and lichens were absent, but a 6 inch deposit of wind-blown sand had accumulated on the west side of the trunk to a distance of about 4 feet. Another oak sheltered a considerable number of individuals, and about 1 foot of sand had accumulated under it to a distance of 8 feet from the trunk. Numerous small trees of *Betula lenta* up to 6 feet in height occupied most of the ground under the northern part of the crown, while a semicircle of individuals of *B. populifolia* up to 6 feet in height was found on the east, south, and west sides at a distance of 8 to 10 feet from the base of the oak. These trees were partially covered by the overhanging branches. This type of circle is common, especially under oaks growing in the *Andropogon-Cladonia* association (fig. 23). In addition to these larger trees, smaller seedlings of black oak were present, with *Andropogon scoparius*, *Trichostema dichotomum*, and *Polygonella articulata*. The ground was thickly covered with acorns, few of which are ever buried deeply enough to allow germination. One other large oak growing in the center of a *Trichostema-Andropogon* community sheltered the following population of species: *Andropogon scoparius*, *Panicum depauperatum*, *P. spp.*, *Carex umbellata*, *Betula populifolia*, *B. lenta*, *Quercus velutina*, *Polygonella articulata*, *Prunus serotina*, *Baptisia tinctoria*, *Chimaphila maculata*, *Vaccinium vacillans*, and *Trichostema dichotomum*.

More important in successional implications than any of the facts mentioned thus far about these isolated oaks is their status as a source of acorn supply for further burial by squirrels and consequent increase of the species in the barren areas. Caches are found as far as 200 feet from the nearest mature tree, often in great numbers. Over twenty-five groups of oaks ranging in age from 1 to 16 years were found within a radius of 100 feet from one isolated tree. The dynamic significance of this fact is apparent. In some areas, however, there has been practically no increase in numbers of oaks in the past 35 years, as shown by photographic records.

#### POLYGONELLA-QUERCUS COMMUNITY

For a number of reasons this community is difficult to describe, since it varies considerably in composition, in density of crown and ground cover, and in its dynamic relations. Its major unifying feature is physiographic, namely, that of its development primarily on wind-blown sand which has accumulated in long ridges or dunes of varying height above a normal soil profile. This sand originates from barren areas at present carrying the *Trichostema-Andropogon* community. A brief summary of the history of these ridges, for which detailed evidence is presented in the section on historical factors, is given now to elucidate the complexities involved in treating this community as a natural unit.

When the townships of North Haven and Wallingford were colonized, the sand plains areas, with their level surfaces and lack of dense forest cover, invited immediate cultivation. They were divided into numerous small fields of a few acres each, following the usual New England method of land allotment. After inclosure with zigzag rail fences, many were put under cultivation, with resultant destruction of all native vegetation except that in the fence rows. This probably consisted of a more or less continuous cover of *Andropogon scoparius*, with occasional forbs, trees, and shrubs of a considerable number of species. The fences afforded perches for birds, with the resultant invasion of such bird-distributed species as *Juniperus virginiana*, *Rhus* spp., *Rubus* spp., *Pyrus arbutifolia*, *Prunus* spp., *Psedera*, *Celastrus*, *Gaylussacia*, *Vaccinium*, etc. Wind-borne seeds and fruits accumulated near them. In addition, the col-

onists planted tree species in the fence rows, such as *Platanus occidentalis*, *Quercus palustris*, *Acer saccharum*, and *A. rubrum*.

Rapid soil depletion soon brought about a cessation of cultivation. Probably most of the fields had been abandoned by 1800. In some of the fields no wind erosion occurred, either before or after abandonment, and these fields were eventually reclaimed by the *Andropogon-Cladonia* association. In many, however, wind erosion was concomitant with cultivation, and probably was a major factor in bringing about abandonment. This erosion resulted in removal of the A horizon. The wind-borne sand was deposited chiefly in and along the fence rows on the south and east sides of each field, sometimes to depths of 8 feet, although usually not more than 3 to 4 feet. Accumulation was not rapid, and in some instances is still continuing. Many of the species present in the rows, especially the grasses and forbs, were not true dune formers, and failed to survive after a few inches of sand had been deposited over the surface. Some of the tree and shrub species have survived to the present day, by repeated sprouting, although the original trunks are gone. This is true of the maples and sycamore. In the main, however, the habitat was now relatively open and bare, with the exception of scattered trees and shrubs, for the fences were soon removed. The annual *Polygonella articulata* probably reached great abundance, which it still maintains. It is the best indicator species of such deposits. The yearly slow accumulation of sand buried numerous wind-borne disseminules of *Betula populifolia* and acorns from occasional trees of *Quercus velutina* which persisted. Burial of the latter by squirrels also occurred, so that an irregular, fairly continuous row of trees developed on these ridges, composed of one or both species. *Betula lenta* came in but was much less important. Such rows, dividing the barrens into numerous small rectangular units, are evident in figure 1. In many of these rows few other species are present besides the trees and *Polygonella*. Where there was an early cessation of sand deposition, moss and lichen mats developed under the trees. These may now form a nearly continuous ground cover for the entire ridge, as shown in figure 17. On one of the largest ridges, that to the east of the large barren 1 in figure 3, the tree canopy is complete, primarily of black oak, with a relatively thick undergrowth of bird-dispersed

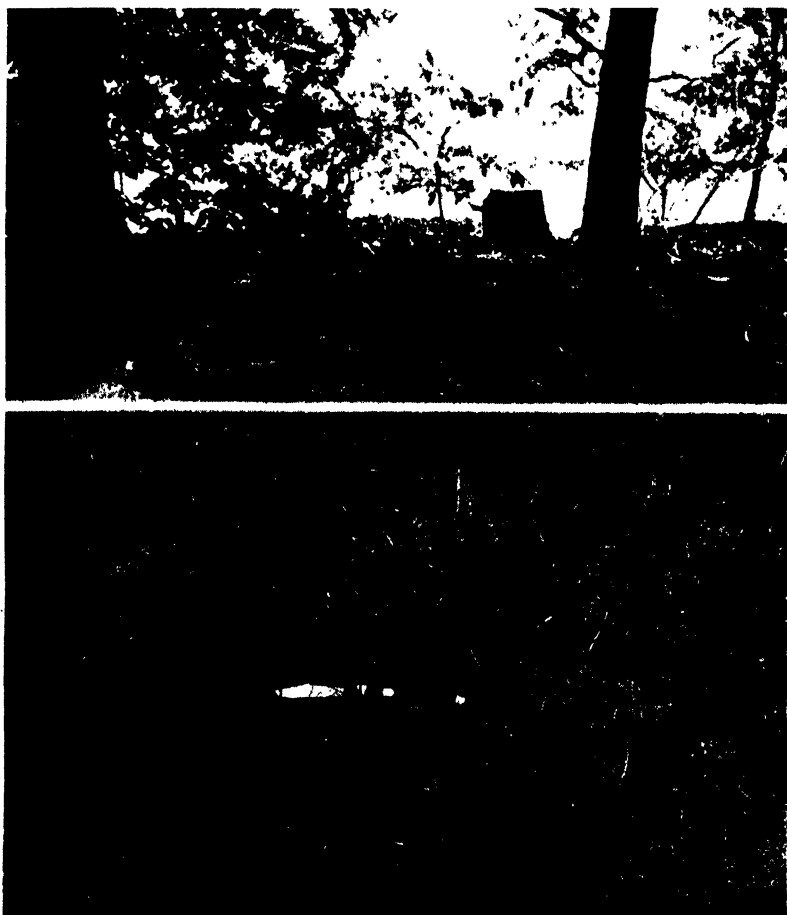
shrubby species, the most important of which is *Rhus toxicodendron*. *Berberis vulgaris*, *Viburnum acerifolium*, and the herbaceous *Smilacina racemosa* and *Polygonatum commutatum* are rare. For the most part the actual soil surface is bare. Another ridge shows a local development of thickets of *Pyrus arbutifolia* and its var. *atropurpurea*, *Rhododendron viscosum*, *Kalmia angustifolia*, *Lyonia ligustrina*, and *Gaylussacia baccata*.



FIG. 16.—Looking eastward along crest of dune, *Polygonella-Quercus* community. Note groups of oaks and mat of *Polytrichum piliferum* just below center of picture. September 19, 1935.

The dune shown in figure 16 is typical for this community. This ridge is approximately 1200 feet long, and varies in width from 45 to 80 feet of wind-deposited material. Average maximum depth of this material above the normal profile is probably 4 feet, although occasional blowouts are lower. As shown, large black oaks are the most important constituents in the tree cover, and are more numerous on the southern lee slope than on the north windward slope. This greater abundance on the lee slope is probably caused by the burial of acorns by sand drift, while the trees on the north have their origin mainly from squirrel caches. Enormous numbers of black oak seedlings are sometimes found on the lee slopes. The total plant popu-

lation of this dune was listed, and the species arranged in groups of primary, secondary, and tertiary importance. The ranking was sub-



FIGS. 17, 18.—Fig. 17 (above), solid mat of *Polytrichum piliferum* partially overgrown by *Cladonia mitis* on wind-deposited sand ridge along an old fence row. Large black oaks and stump of *Juniperus virginiana* in background. Mat carries seedlings of *Andropogon scoparius* and *Juniperus virginiana* and one plant of *Asclepias amplexicaulis*. July 12, 1935. Fig. 18 (below), *Carex pennsylvanica* and *Lecidea uliginosa* on wind-deposited sand ridge. Small amounts of *Cladonia strepsilis* and *C. cristatella* also present. September 19, 1935.

jective, but based chiefly on a combined estimate of abundance and cover. Species of primary importance, arranged in taxonomic se-

quence, are *Lecidea uliginosa*, *Polytrichum piliferum*, *Betula populi-folia*, *Quercus velutina*, *Polygonella articulata*, and *Platanus occidentalis*. Species of secondary importance are *Cladonia strepsilis*, *Dicranella heteromalla*, *Andropogon scoparius*, *Panicum depauperatum*, *P. tsugetorum*, *P. columbianum*, *Prunus serotina*, and *Trichostema dichotomum*. Species of tertiary importance, usually rare, are *Diploschistes scruposus parasiticus*, *Lecidea granulosa*, *Cladonia cristatella*, *C. coniocraea*, *Geaster* sp., *Juniperus virginiana*, *Panicum auburne*,

TABLE 3

ABUNDANCE BY METER QUADRATS IN SOCIETY OF *LYSIMACHIA QUADRIFOLIA*

SPECIES	QUADRAT			
	1	2	3	4
<i>Cladonia mitis</i> . . . . .			♂*	
<i>C. bacillaris</i> . . . . .			♂	♂
<i>C. cristatella</i> . . . . .		♂		♂
<i>C. strepsilis</i> . . . . .			♂	
<i>Cetraria islandica</i> . . . . .		♂		
<i>Andropogon scoparius</i> (seedling) . . . . .		4	2	5†
<i>Panicum depauperatum</i> (seedling) . . . . .	18	10	6	8
<i>P. spp.</i> (seedlings) . . . . .	5	3	1	5
<i>Betula populifolia</i> (seedling) . . . . .		1		
<i>Polygonella articulata</i> . . . . .	13	18	24	23
<i>Hypericum gentianoides</i> . . . . .				1
<i>Lysimachia quadrifolia</i> . . . . .	29	36	47	57
<i>Trichostema dichotomum</i> . . . . .	61	4	14	35

\* ♂ Present but unimportant. Date of counting: August 20, 1935. Average height of *Lysimachia* 12 cm., maximum height, 20 cm.

*P. commonsianum*, *Danthonia spicata*, *Cyperus filiculmis macilentus*, *Cypripedium acaule*, *Sassafras variifolium*, *Rhus toxicodendron*, *Acer rubrum*, *Hypericum gentianoides*, *Helianthemum majus*, *Monotropa uniflora*, *Vaccinium corymbosum*, *Fraxinus americana*, and *Asclepias amplexicaulis*.

Other species not mentioned thus far on the sand ridges, which may have considerable local importance, are *Carex umbellata*, *C. pennsylvanica*, and *Helianthemum propinquum*. Low caespitose clumps of the first species, often a foot or more in diameter, occur on such deposits, usually on fairly stable surfaces near trees. The second species is an excellent stabilizer and may form extensive carpets on these ridges, readily invaded by *Lecidea uliginosa* (fig. 18). The

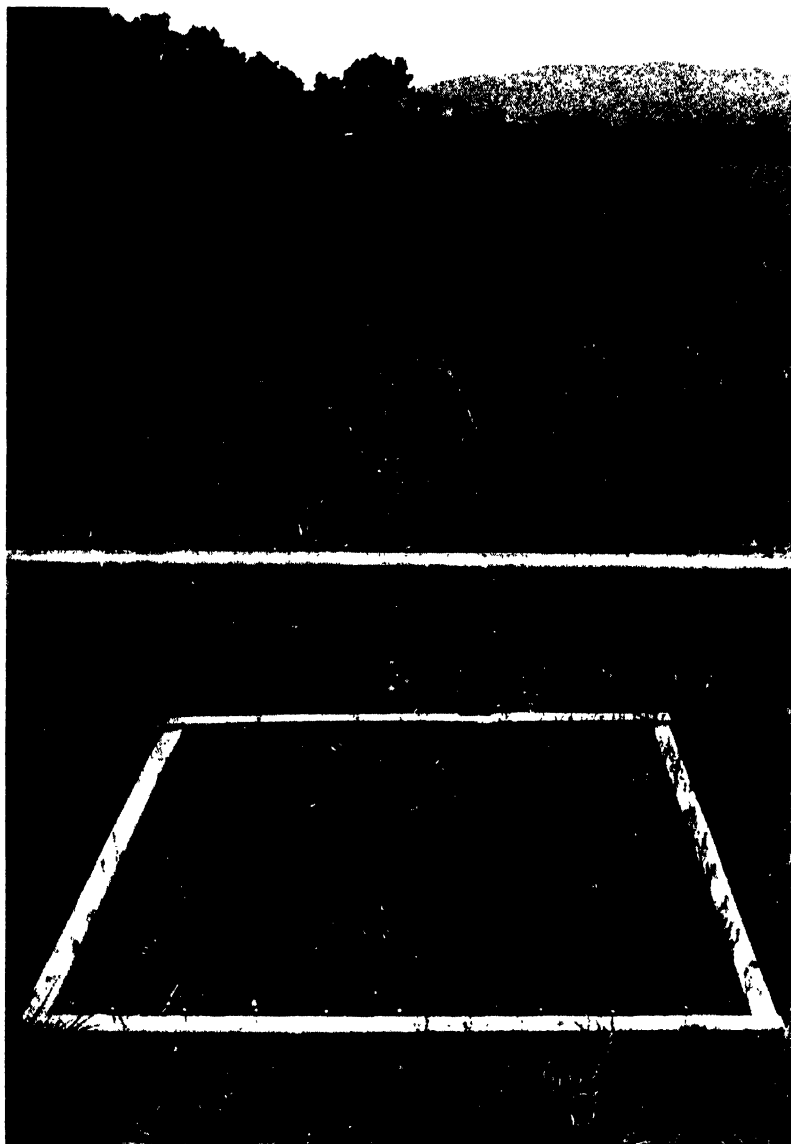


third species develops large mats on these deposits as well as on unweathered sand on the floor of sand pits. *Lysimachia quadrifolia* becomes established on the ridges under oaks and spreads rapidly by means of vigorous vegetative propagation, although flower and fruit production is limited and shoots of the plants are small, often not more than 12 cm. high. Abundance in meter quadrats in such a society around one tree is given in table 3.

#### ANDROPOGON-CLADONIA ASSOCIATION

This community is undoubtedly worthy of association ranking. It covers a larger area than any other type of natural plant community on the sand plains. Each stand shows the same essential grouping of species, occurring almost exclusively on areas with a normal soil profile, which experienced early cultivation and were then abandoned. General views of this community are seen in figures 6 and 19. It probably resembles original vegetation on these areas later eliminated except along the fence rows during the period of cultivation. The successional sequence following abandonment may be inferred.

GRASSES AND FORBS.—The first abundant invaders were probably annuals, especially *Hypericum gentianoides*. In some of the larger fields, isolated islands of this species are still found (fig. 6). However, grasses, especially *Andropogon scoparius*, *Panicum depauperatum*, and a number of species of *Panicum* in the sections Lanuginosa, Columbiana, and Sphaerocarpa, soon invaded these open fields from their fence row refuge, developing scattered bunches of varying size which in time occupied fully 25 per cent of the soil surface. Numerous forbs entered at the same time or later. This invasion continues to the present time. Many species of forbs, rare in the *Andropogon-Cladonia* association at present or restricted to their undisturbed habitat in old fence rows from which the rails were long since removed, were doubtless occasional or abundant in the pre-colonial grassland. Among such forbs are *Fragaria virginiana*, *Potentilla canadensis*, *P. pumila*, *Lupinus perennis*, *Tephrosia virginiana*, *Desmodium rigidum*, *Linum virginianum*, *Lechea intermedia*, *L. villosa*, *Viola pedata*, *V. fimbriatula*, *Apocynum cannabinum*, *Stachys hysso-pifolia*, *Pycnanthemum virginianum*, *Eupatorium hyssopifolium*, *Chry-*



FIGS. 19, 20.—Fig. 19 (above), general view of *Andropogon-Cladonia* association, with scattered young black oaks and gray birch. Large trees to left are *Betula lenta* in an old fence line. August 19, 1935. Fig. 20 (below), meter quadrat in *Andropogon-Cladonia* association, showing mat of *Polytrichum piliferum* and lichens completely covering the ground between the grass clumps. Seedlings of the grass are present on this mat. September 19, 1935.

*sopsis falcata*, *Solidago bicolor*, *S. juncea*, *S. odora*, *S. rugosa*, *S. tenuifolia*, *Aster dumosus*, *A. vimineus*, *Sericocarpus asteroides*, *Antennaria neglecta*, *Anaphalis margaritacea*, *Gnaphalium polycephalum*, *Helianthus divaricatus*, *Krigia virginica*, and *Hieracium scabrum*. Many of these are completely restricted to ancient fence lines, or are found rarely in the *Andropogon-Cladonia* association where the community developed on the sands of highest colloidal content. *Panicum meridionale*, *P. sphacrocarpon*, *P. scribnerianum*, *Agrostis perennans*, *Danthonia spicata*, *Eragrostis spectabilis*, *Poa compressa*, *Carex brevior*, *C. merritt fernaldii*, *Juncus tenuis*, and *Luzula campestris multiflorus* are constituents of this association only on such sands, although some occur in other communities.

Certain forbs and grassy plants have been more successful in re-establishing themselves, and are not uncommon in most stands of the association, showing high frequency and abundance although cover values are low. These species include *Cyperus filiculmis macilentus*, *Carex muhlenbergii*, *Baptisia tinctoria*, *Desmodium canadense*, *Lespedeza capitata*, *Helianthemum canadense*, *Lysimachia quadrifolia*, *Asclepias amplexicaulis*, *A. verticillata*, *Solidago nemoralis*, *S. graminifolia nuttallii*, *Aster linariifolius*, and *Artemisia caudata*.

In certain areas within the *Andropogon-Cladonia* association, which have been plowed recently and were again abandoned, *Panicum depauperatum* now forms the major portion of the vegetation. On finer sands it covers as much as 60 per cent of the ground. In spaces between grass bunches, *Hypericum gentianoides* and *Lecidea uliginosa* may occur in abundance. In other areas of similar history *Andropogon scoparius* is gaining almost complete control, often associated with a greater abundance of *Lespedeza capitata* than is typical for the mature association. One area, abandoned 10 years ago immediately after plowing, showed a relatively vigorous development of these species, with *Lecidea uliginosa*, *Baeomyces roseus*, *Cladonia papillaria*, *C. cristatella*, and *C. strepsilis* coming in on the ground. Other areas showed mixed stands of the two grasses, one within 9 years after abandonment. Causes for the differences in these pioneer stages of this subsera have not been investigated, but may be related to differential production, viability, dispersal, and

ecesis of the disseminules of the plants mentioned. It would be unwise to assume that these communities represent an exact duplication of the pioneer stages which preceded the mature association in its first development. Longer periods of cultivation at that time would have vitiated most of the propagules in the soil, and invasion would have been largely a result of seed dispersal from undisturbed fence row communities.

LICHENS AND MOSSES.—More important than any of the forbs or secondary grasses in the ground covered and in their effect on the habitat are the moss and lichen members of the *Andropogon-Cladonia* association, which occupy the ground completely between the bunches of grass (fig. 20). These probably invaded abandoned fields when the grass bunches had become numerous enough to afford some protection from wind sweep. This sequence may be inferred from the current invasion of grass, moss, and lichens into isolated *Hypericum* communities. *Polytrichum piliferum*, *Lecidea uliginosa*, and *Cladonia papillaria*, especially the first two species, seem to be pioneers, invading singly or simultaneously. The moss is probably the only one capable of spreading alone very far from grass protection. Other lichens then invade the area, both upon and between plants of the pioneer species. *Cladonia strepsilis* establishes itself readily on the crustose thallus of *Lecidea uliginosa*, and in the moss mats, and is usually next in the succession. A great number of species may follow, such as *Baeomyces roseus*, *Lecidea granulosa*, *Diploschistes scruposus parasiticus* (usually on *Cladonia papillaria*), *C. bacillaris*, *C. chlorophaea*, *C. clavulifera*, *C. coniocraea*, *C. mateocyatha*, *C. pleurota*, and *C. squamosa levicorticata rigida*. Finally the fruticose forms of *Cladonia mitis* and *C. uncialis* appear. Arrival of *Geaster* sp. and *Polytrichum commune* almost completes the list of macroscopic non-vascular plants which are usually found in the association as typically developed. During invasion, *Lecidea uliginosa* usually loses ground to the invaders, *Polytrichum piliferum* does in part, but *Cladonia papillaria* maintains its strong position, and with *C. strepsilis* may be regarded as the most abundant lichen in the association as a whole.

Many lichen species are represented by several forms. This is especially true of *Cladonia cristatella*, a species not yet mentioned. It

has a unique role in the community. Correlation of the invasion of this species with beginning decay of plants which invade sterile abandoned fields in North Carolina was noted by GRAY (31). Its appearance as the form *C. c. abbreviata* in the center of *Andropogon* clumps in the *Trichostema-Andropogon* community has already been noted. The persistent vitality of these grass clumps, their relative youth, and the accumulation of sand within them are not conducive to lichen development. Nevertheless in the *Andropogon-Cladonia* association many grass clumps of relatively early establishment have died, and show various stages of disintegration and decay. *C. c. abbreviata* is common on leaves of grasses in early stages of decay, but on clumps reduced to broken culms and fragments of leaf sheaths the species is usually represented by f. *vestita*. Scarcely a dead clump of this character lacks the lichen. Other lichens, especially *Lecidea uliginosa*, *Cladonia papillaria*, and *C. strepsilis*, also extend their thalli over these dead clumps, and the other species mentioned may soon follow. In the meantime grass seedlings, prospering on the original lichen and moss mats, mature and eliminate the lower species through overgrowth and displacement. Thus a more or less continuous cycle occurs within a relatively permanent community.

Quadrat data for two stands of the *Andropogon-Cladonia* association are given in table 4. The data are based on twenty quadrats in each stand, spaced every 50 feet along straight lines. Stand 2 is shown in figure 19. BLIZZARD (3) and CONARD (14) have described similar communities on Long Island, New York.

Table 4 summarizes the composition of the *Andropogon-Cladonia* association as developed on the coarsest sands with low colloidal content, of the order indicated in table 11. Stands developed on the finer sands not only may show many or all of the forbs listed on page 246, but may also have a higher cover value for *Andropogon*, often approaching 50 per cent, with consequent lower values for lichens and moss.

PERSISTENCE OF ASSOCIATION.—Persistence of this association for approximately 150 years is probably due to two causes. Dispersal of disseminules of dominant plants of higher types of associations has been slow and scanty into many of the areas, especially into those of larger extent, since the source of supply was relatively

limited. Those woody plants which became established were subjected to repeated fires, which tended to eliminate or to keep them

TABLE 4

## METER QUADRAT DATA FOR THE ANDROPOGON-CLADONIA ASSOCIATION

SPECIES	STAND 1		STAND 2	
	F%*	C%	F%	C%
<i>Diploschistes scruposus parasiticus</i> ...	40	1.0	25	2.0
<i>Lecidea granulosa</i> .....	50	4.0	45	4.0
<i>L. uliginosa</i> .....	90	9.5	85	8.0
<i>Baeomyces roseus</i> .....	40	0.5	25	1.5
<i>Cladonia mitis</i> .....	95	10.5		
<i>C. papillaria</i> .....	85	7.5	90	8.5
<i>C. pleurota</i> .....	15	0	40	0.5
<i>C. cristatella</i> .....	100	8.5	75	5.5
<i>C. squamosa levicorticata rigida</i> .....	10	0	5	0
<i>C. clavulifera</i> .....	15	0	15	0
<i>C. mateocyatha</i> .....			70	9.0
<i>C. coniocraea</i> .....	15	0		
<i>C. strepsilis</i> .....	90	12.0	100	20.0
<i>Geaster</i> sp.....	25	0	10	0
<i>Polytrichum piliferum</i> .....	95	10.0	100	16.5
<i>P. commune</i> .....	5	0.5	30	4.0
<i>Andropogon scoparius</i> .....	100	22.5	100	32.5
<i>Andropogon scoparius</i> (dead).....	90	13.5	95	10.0
<i>Panicum depauperatum</i> .....	90	8.0	80	5.0
<i>P. spp.</i> .....	25	2.0	90	5.5
<i>Carex umbellata</i> .....			5	0.5
<i>C. pennsylvanica</i> .....			10	0
<i>Betula populifolia</i> (seedling).....			5	0
<i>Quercus velutina</i> (seedling).....	5	0		
<i>Polygonella articulata</i> .....	25	0	20	0
<i>Rubus villosus</i> .....	20	1.5	5	0
<i>Prunus serotina</i> (seedling).....	5	0		
<i>Cassia nictitans</i> .....			5	0
<i>Baptisia tinctoria</i> .....	10	2.5		
<i>Lespedeza capitata</i> .....			20	0.5
<i>Hypericum gentianoides</i> .....	15	0	5	0
<i>Helianthemum propinquum</i> .....	5	0.5		
<i>Lysimachia quadrifolia</i> .....	20	2.0	50	3.5
<i>Trichostema dichotomum</i> .....	5	0		
<i>Aster linariifolius</i> .....	15	2.0	10	1.0

\* F, frequency; C, cover; 0, cover value practically none. Dates of counting: stand 1, June 27, 1935; stand 2, June 29 and July 16, 1935.

suppressed. Recently fires have been kept largely under control, and invasion of this association by a number of woody plants is occurring.

WOODY MEMBERS.—*Rubus villosus* has a high value for presence in

the stands of this association, and should be considered as a normal and old constituent of the community, although its frequency in any particular stand may be low. It seems to have little dynamic significance. The same is true of the much rarer species, *Rubus semisetosus*. Another woody plant, *Myrica asplenifolia*, is also common. Its open growth in this habitat fails to disturb the dominance of *Andropogon* to any marked extent, although it does tend to eliminate lichens and moss. Through vegetative propagation, it may extend over several hundred square yards in a few years. Other woody invaders have considerable significance. Since many of these tend to invade the association directly from positions in ancient fence lines which traverse many stands of the association, these fence row communities will be described briefly. The fences were removed long ago.

FENCE LINE RELICTS.—Long strips, 10 to 15 feet wide, with an uncultivated and undisturbed normal soil profile, are the substrata for the fence row communities. Species present are mainly relicts of the original vegetation and bird-distributed species which came in following erection of fences. In some cases the latter are lacking, and the vegetation constitutes an *Andropogon* grassland, in which many of the forbs named on page 246 are found. *Andropogon* cover may be as high as 90 per cent, lichens and moss being relatively unimportant or lacking. The total population in fence lines traversing stand 2 (3 in figure 2) of the *Andropogon-Cladonia* association consisted of: *Lecidea granulosa*, *L. uliginosa*, *Baeomyces roseus*, *Cladonia mitis*, *C. papillaria*, *C. bacillaris*, *C. pleurota*, *C. cristatella*, *C. squamosa levicorticata rigida*, *C. mitrula*, *C. clavulifera*, *C. mateocyatha*, *C. coniocraea*, *C. strepsilis*, *Dicranella heteromalla*, *Polytrichum piliferum*, *P. commune*, *Juniperus virginiana*, *Andropogon scoparius*, *Panicum depauperatum*, *P. tsugetorum*, *P. columbianum*, *Agrostis perennans*, *Danthonia spicata*, *Carex pennsylvanica*, *C. sp.*, *Myrica carolinensis*, *Betula lenta*, *B. populifolia*, *Quercus velutina*, *Q. ilicifolia*, *Fragaria virginiana*, *Potentilla pumila*, *P. canadensis*, *Rubus villosus*, *Prunus serotina*, *Baptisia tinctoria*, *Lespedeza capitata*, *Rhus glabra*, *R. copallina*, *R. toxicodendron*, *Psedera quinquefolia*, *Helianthemum propinquum*, *H. canadense*, *Viola pedata*, *Nyssa sylvatica*, *Chimaphila maculata*, *Gaylussacia baccata*, *Vaccinium vacillans*,

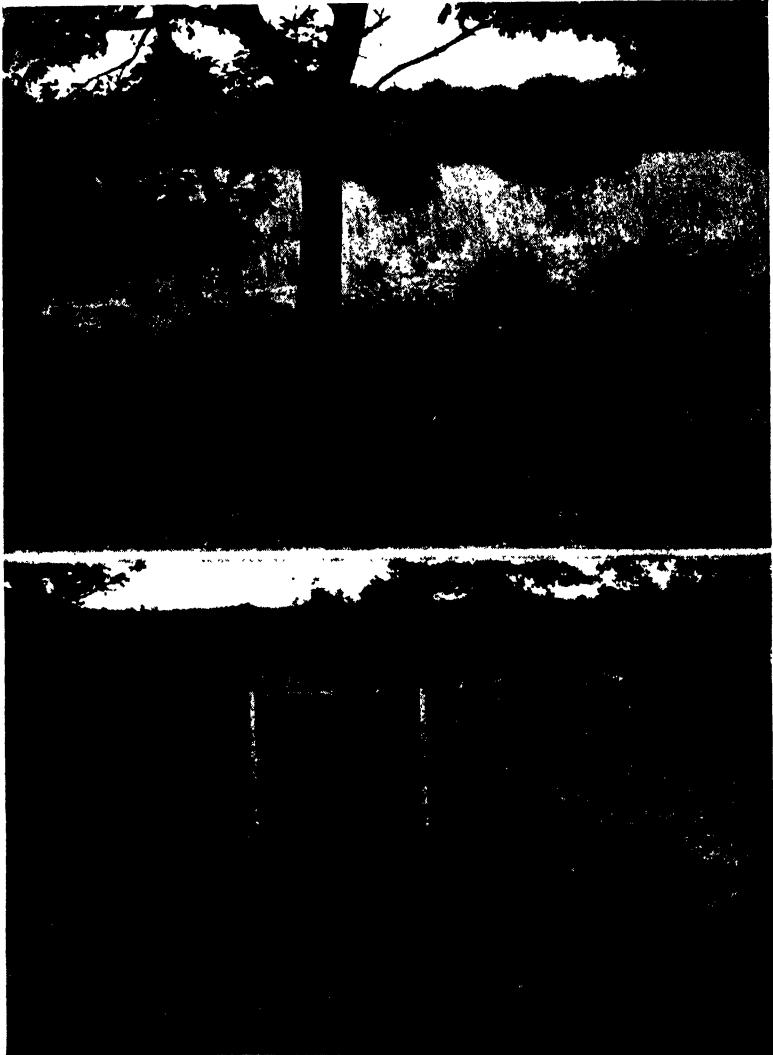
*Lysimachia quadrifolia*, *Asclepias amplexicaulis*, *Chrysopsis falcata*, *Solidago bicolor*, *S. juncea*, *S. odora*, *S. nemoralis*, *Aster dumosus*, *A. linariifolius*, and *Antennaria neglecta*. Space does not permit a detailed description of the many ways in which these species may be grouped. Their abundance and frequency change markedly from place to place, so that no accurate quantitative survey is possible. It should be noted that *Dicranella heteromalla* and *Danthonia spicata* are characteristic species in the ground cover under isolated trees with spreading crowns, and are practically restricted to such habitats.

Other woody species which may be found in these ancient fence lines, and some of which invade the *Andropogon-Cladonia* association, are: *Pinus rigida*, *Juniperus communis*, *Juglans cinerea*, *Corylus americana*, *Castanea dentata* (sprouts), *Quercus palustris*, *Sassafras variifolium*, *Platanus occidentalis*, *Pyrus arbutifolia*, *Amelanchier stolonifera*, *Rubus* sp., *Prunus pennsylvanica*, *Robinia pseudoacacia*, *Rhus typhina*, *Celastrus scandens*, *Acer saccharum*, *A. rubrum*, *Rhododendron viscosum*, *Kalmia angustifolia*, *Lyonia ligustrina*, *Vaccinium pennsylvanicum*, and *V. corymbosum*.

WOODY INVADERS.—Of all the woody species named in the two lists above, very few are of value in bringing about a destruction of the *Andropogon-Cladonia* association. Most of them, by reason of low frequency on the sand plains as a whole and resultant scanty seed dispersal, or through apparent inability to invade this closed association except in the company of certain pioneer trees and shrubs, need not be considered further in any detail. Direct invaders of considerable importance are *Pinus rigida*, *Juniperus virginiana*, *Myrica carolinensis*, *Betula populifolia*, *Quercus velutina*, *Q. ilicifolia*, *Prunus serotina*, *Robinia pseudoacacia*, *Gaylussacia baccata*, and *Vaccinium vacillans*. The majority of these species are bird-distributed. Birch and pine produce their wind-carried seeds in abundance, and invasion of the oaks is probably brought about primarily through activities of squirrels.

The three shrubs, *Myrica carolinensis*, *Gaylussacia baccata*, and *Vaccinium vacillans* (fig. 22), form low dense thickets which effectually displace the *Andropogon-Cladonia* association, and in turn will probably give way to trees, especially oaks. The birch, red





FIGS. 21, 22.—Fig. 21 (above), three black oaks in *Andropogon-Cladonia* association, showing squirrel cache origin. Bird-dispersed *Vaccinium corymbosum*, *V. vacillans*, and *Gaylussacia baccata* present under the crown. Invasion of *Pinus rigida* into the lichen-grassland association seen in background. August 31, 1935. Fig. 22 (below), clump of *Vaccinium vacillans*, 9×10 feet, in *Andropogon-Cladonia* association. Small gray birches and large black oaks scattered in background in same community. September 7, 1935.

cedar, and black locust, even at maturity, usually form pure or mixed open stands in which the ground cover consists primarily of species in the original community. The importance of *Rubus* spp. and *Carex muhlenbergii* may be increased somewhat, especially in stands of black locust. *Aster linariifolius* occurs in extensive societies under red cedar. As yet there is little evidence of the invasion of species characteristic of later successional stages which follow the red cedar-gray birch association (38) in southern New England. *Prunus serotina* is usually found only as shrubby isolated individuals. *Quercus ilicifolia* is likewise uncommon as an invader of the *Andropogon-Cladonia* association. Extensive, nearly pure stands of this species which occur in certain portions of the sand plains were not studied in detail. They were thought to represent a phase of the original vegetation which escaped destruction through cultivation, although subjected to fire and partial cutting. Such communities occurred on the New Haven plain at the time of white settlement (20).

*Quercus velutina* is one of the two most important species which invade the *Andropogon-Cladonia* association. Although mass invasion may occur, the usual appearance of the species is as isolated individuals (fig. 23) or in close groups (fig. 21). In any case, dispersal of the species is probably brought about through burial of acorns by squirrels as previously described in detail, there being slight opportunity otherwise for burial and germination. The common occurrence of grouped individuals would indicate this origin. Stumps of one group of three cut early in 1932 were 34, 21, and 14 inches in diameter, 33 inches above the ground, and showed seventy-eight growth rings. An age of 85 to 90 years is indicated. In spite of size differences, no marked suppression in yearly increment was apparent in any stump. The importance of these isolated individuals and groups is essentially the same as in the barrens communities, namely, as sources of disseminules for further dispersal, and as colonization centers for other species. The occurrence of *Betula populifolia* in a circle under their canopies (fig. 23) has been noted previously. This phenomenon is very common. Bird-distributed species are also found under them shortly after they have attained a moderate size. The following species were found under the trees seen in figure 21,



FIGS. 23, 24.—Fig. 23 (above), circle of young gray birches under a large isolated black oak in *Andropogon-Cladonia* association. September 22, 1935. Fig. 24 (below), invasion of *Andropogon-Cladonia* association by *Pinus rigida*. Smaller pines in center are probably offspring of large tree on right. A black oak, originally isolated in lichen-grass association, is on the left. August 20, 1935.

which were only 20 feet tall with a 20 foot crown spread: *Cladonia mitis* (including *C. m. soralifera*),<sup>8</sup> *Cetraria islandica* (other lichens not listed), *Polytrichum piliferum*, *Pinus rigida* (seedling), *Andropogon scoparius*, *Betula populifolia*, *Prunus serotina* (seedlings), *Baptisia tinctoria*, *Gaylussacia baccata*, *Vaccinium vacillans*, and *V. corymbosum*. Other species which have been found under such trees are *Juniperus virginiana*, *J. communis*, *Carex debilis rudgei*, *Pyrus arbutifolia*, and *Vaccinium pennsylvanicum*. Barring further fires, oak forests might supplant the *Andropogon-Cladonia* association directly through increase in abundance of the species around these isolated pioneers, although the only stands of black oak worthy to be called forests which now occur on the sand plains probably did not arise in this way. Their occurrence and significance will be discussed later.

#### PINUS RIGIDA ASSOCIATION

The forests of pitch pine in the New Jersey Pine Barrens have been studied repeatedly by botanists and ecologists. Similar forests, although poorer floristically because of their postglacial origin, were extensively developed on many of the sandy level terraces in southern New England at the time of white settlement (9, 20). No historical evidence has been found to indicate that such forests formed an important part of the original vegetation on the North Haven-Wallingford plains. At present, with the exception of a few sporadic individuals, pitch pine occurs only in the stands seen at 1 and 5 in figure 2. Nevertheless, with the possible exception of black oak, this species seems to have more successional significance than any other tree species now growing on the plains.

These stands of pitch pine differ somewhat in character. Those shown at 5 in figure 2 are open, with an undergrowth composed of species belonging to the *Andropogon-Cladonia* association, in which one very open thicket of *Myrica asplenifolia* covers several hundred square yards. The open stand of trees is caused in part by fire, and occasional selective and clear cutting, and in part by the recent and sporadic invasion of the pine. Soil profile studies indicate a pre-colonial grassland in most of the area, and it is known that the larger

<sup>8</sup> The first published report of the occurrence of this form in North America was based upon specimens secured from this station (24).

portion of the area south of the diagonal road was cultivated and abandoned.<sup>9</sup> Portions of this stand, however, may represent relicts of an original pitch pine forest, much disturbed by cutting and fire. Barring the occurrence of extensive original forests of this species in the area, individuals in the living stands may be lineal descendants of trees which were found, probably scattered, in oak forests. Such forests still persist, although modified by cutting, on the islands of till in the sand plain, and on the ridge of till-covered sandstone to the east. One island of till is found almost in the center of the present pine stand, and another is near the southern end.

Relationships of the pitch pine stand seen at 1 in figure 2 are more clear cut. This stand, largely developed in the past 45 years, has been invading an *Andropogon-Cladonia* association in which isolated oaks of various sizes and ages occur. The method of establishment is indicated in figures 21 and 24. Isolated individuals with their low crowns and spreading branches first become established in the grass-lichen community, but the prolific production of the large-winged seeds at ages of 10 years or less results in a mass invasion into the lower community. The general invasion has been from the southwest corner of the stand. Here are found a few large trees, which show forty-five to sixty-five growth rings at breast height and bear persistent stubs of large lower branches (fig. 26), indicating an origin in the open. Most of these trees are growing on a grassland type of soil profile, which shows former cultivation in the persistence of furrow ridges. Near the margin of the terrace, however, the profile indicates forest development of considerable duration, and the old pines which occur may be lineal descendants of members of an original forest which occupied the same location.

Shade and thick accumulation of needles in the developing pine stand bring about rapid elimination of most of the herbaceous and lichen species of the preceding association. *Polytrichum piliferum* disappears, but *P. commune* may develop luxuriant carpets (fig. 25). *Cladonia cristatella* persists on the dead, and invades the dying, grass clumps. When these have been decomposed, it can come in on fallen wood, and thus persist in the pine community. *Cladonia chlorophaea* and *C. bacillaris* also have a rather high frequency in the pine stand,

<sup>9</sup> Family history of residents in the vicinity.



FIGS. 25, 26.—Fig. 25 (above), young pine stand, about 25 years old, showing suppression through overstocking. Ground cover mainly of pine needles, with mats of *Polytrichum commune* and numerous seedlings of black oak. September 22, 1935. Fig. 26 (below), open-grown pine, 45 years old DBH, surrounded by dense growth of young black oaks, gray birch, and chestnut sprouts. Base of pine surrounded by clump of *Vaccinium vacillans*, which perhaps originated through bird dispersal while the tree was isolated. September 22, 1935.

but with low abundance, and *C. caroliniana dilatata* and *Baeomyces roseus* have been collected locally. Few other lichens persist in or have invaded this young pine stand. *Vaccinium vacillans* and *V. pennsylvanicum* persist beneath the crowns (fig. 26), and extend outward from the formerly isolated mature pines and oaks. Humus-re-

TABLE 5  
QUADRAT DATA FOR THE PINE-OAK TRANSITION COMMUNITY

SPECIES	F%*	A	AVERAGE HT.	AVERAGE DBH (IN.)	TOTAL BASAL AREA (SQ. IN.)
<i>Pinus rigida</i> .....	19	26			
12 ft. plus.....	12	12	30 ft.	6	457
6-12 ft.....	3	3	8 ft.	1	2.36
1-2 ft.....	2	3			
6-12 in.....	1	1			
0-6 in.....	3	7			
<i>Quercus velutina</i> .....	53	88			
12 ft. plus.....	4	4	26 ft.	5	119
6-12 ft.....	10	14			
3-6 ft.....	7	7			
2-3 ft.....	7	8			
1-2 ft.....	7	7			
6-12 in.....	7	8			
0-6 in.....	30	40			
<i>Betula lenta</i> .....	1	1	2 ft.		
<i>B. populifolia</i> .....	3	3	7 ft.		
<i>Fagus grandifolia</i> .....	1	1	9 ft.		
<i>Prunus serotina</i> .....	3	3	7 in.		
<i>Acer rubrum</i> .....	1	1	2 in.		
<i>Cladonia bacillaris</i> .....	1				
<i>C. cristatella</i> .....	41				
<i>C. chlorophaea</i> .....	5				
<i>Polytrichum commune</i> .....	3				
<i>Andropogon scoparius</i> .....	7	7			
<i>Cypripedium acaule</i> .....	15	53			
<i>Chimaphila maculata</i> .....	11	19			

\* F, frequency; A, abundance; DBH, diameter breast high. Date of counting: August 1, 1932. Data based on 100 adjoining quadrats in a 10X10 meter plot.

quiring species, such as *Monotropa hypopitys* and *Cypripedium acaule*, also come in. The latter is frequent and abundant wherever the stand approaches maturity. *Chimaphila maculata* is not rare. Other than these, there is little undergrowth except that of tree species in the young and mature pine woods. The original overstocked condition causes suppression of great numbers of pines (fig. 25), for the species reproduces poorly under its own canopy. However, very fa-

vorable seed bed conditions have been created for oaks. Barring fires, considerable numbers of black oak seedlings appear on the forest floor (fig. 25) which as acorns became scattered from formerly isolated mature individuals of this species or from the narrow oak forest at the margin of the terrace to the west. If not disturbed, this indicates an eventual extinction of the pine forest and its replacement by one of oak (fig. 26). Quadrat data for a 100 square meter plot in the transitional region are given in table 5. Similar dynamic tendencies are at work in the pine stands on the east side of the plains, but more prevalent disturbance by cutting and fire hinders completion of the succession.

#### QUERCUS SPP. ASSOCIATION

Oak forests within the sand plains area under consideration are mostly limited to the islands of till mentioned on p. 258, to small strips within and bordering the ravines of transverse streams, and to a continuous marginal strip on the west side of the plain. The name forest has not been applied to those aggregations of oaks discussed in many of the preceding sections, even when the canopy is nearly complete, since the undergrowth in such communities as yet bears little resemblance and "sociological affinity" to that found in the typical xerophytic oak forest as developed in Connecticut. In time similarities will probably arise, but for the present purpose there is nothing to be gained in regarding such communities as early stages in the development of the forests now present.

The forests on the till islands will not be mentioned further since they cannot be considered as members of the sand plains succession. The marginal and ravine forests, however, occur on deposits of deep sand and are well above the influence of the ground water table. In part they probably represent relicts of a similar type of original vegetation, which have been disturbed only by fire and cutting during white occupation, although at least one of them occurs on a grassland soil profile. It is unlikely that the isolated point of land on which the soil pit was dug had ever been under cultivation. Therefore, an invasion of the oak forest into original grassland must be assumed, although the time of invasion cannot be determined. Such forests have also replaced the *Andropogon-Cladonia* association local-



ly by direct mass invasion where they lie adjacent to it. As indicated, the pine association is also threatened.

In their present topographic location, these forests adjoin the centers of mesism found in ravine, swamp, and protected slope. Their original development and persistence have resulted in part from the ameliorating action of such centers upon evaporation rates, and from the protection afforded from fires, which undoubtedly swept over the plains as a whole long before white settlement. Without such fires it is probable that this type of forest would have been widely developed on the plains, instead of the grassland. It might even have reached the degree of mesophytism represented by the climatic climax. Since none of the present forests on the level terrace do, it is much easier to believe that such a hypothetical forest would be an edaphic climax community dominated by black oak, with scattering representatives of more mesophytic trees.

Although none of the oak forests were analyzed statistically, notes were made on their composition. Besides the dominant black oak, both *Quercus borealis maxima* and *Q. alba*, especially the latter, are not uncommon. Other tree species which may occur sporadically in the nearly complete canopy are *Betula lenta*, *B. populifolia*, *B. papyrifera*, *Fagus grandifolia*, *Sassafras variifolium*, and *Prunus serotina*. The shrub layer consists only of extremely scattered individuals of *Juniperus communis*, *Castanea dentata* sprouts, *Quercus ilicifolia*, *Gaylussacia baccata*, and *Vaccinium vacillans*. The ground layer is also incomplete. Aside from seedlings of these plants, the following species have been recorded: *Cladonia cristatella* (other lichens also occur, although not in abundance), *Leucobryum glaucum*, *Polytrichum commune*, *P. ohioense* (other mosses rare), *Lycopodium tristachyum*, *Carex pennsylvanica*, *Panicum* spp., *Agrostis hiemalis*, *A. perennans*, *Danthonia spicata*, *Luzula campestris multiflorus*, *Maianthemum canadense*, *Cyripedium acaule* (common), *Rubus* spp., *Desmodium nudiflorum*, *Rhus toxicodendron*, *Acer rubrum* seedlings, *Psedera quinquefolia*, *Chimaphila maculata*, *C. umbellata*, *Monotropa uniflora*, *M. hypopitys*, *Lysimachia quadrifolia*, *Trientalis americana*, *Melampyrum lineare*, and *Solidago odora*. Very few of these species are characteristic of climatic climax forests in Connecticut. Distant views of the oak forests are seen in figures 10 and 13.

## MIXED MESOPHYTIC FOREST ASSOCIATION

The transition, down a relatively steep westward-facing sandy slope, from the level westward margin of the terrace, often covered with a forest of the type described, to the red maple swamp forests of old oxbows and the cultivated fields and floodplain forests of the lower Quinnipiac River valley, has been mentioned before. This slope is frequently covered with a forest which may approach the climatic climax type in mesophytism and in composition. Besides the species listed under the oak association, the following vascular plants have been recorded from this mesophytic forest: *Polystichum acrostichoides*, *Aspidium noveboracense*, *A. spinulosum*, *Dicksonia punctilobula*, *Lycopodium obscurum*, *L. clavatum*, *Medeola virginiana*, *Carpinus caroliniana*, *Betula lutea*, *Liriodendron tulipifera*, *Benzoin aestivale*, *Hamamelis virginiana*, *Rubus hispidus*, *Aralia nudicaulis*, *Rhododendron nudiflorum*, *Vaccinium corymbosum*, *Fraxinus americana*, *Epifagus virginiana*, *Mitchella repens*, *Viburnum dentatum*, *Solidago caesia*, *Aster divaricatus*, *Prenanthes* sp., and *Hieracium paniculatum*. It is unnecessary to point out the mesic and climax nature of some of these species. However, only the most ardent adherents to the monoclimate theory would contend that forests of this type would have developed extensively on the level exposed terrace.

## Planting experiments

It was deemed advisable, in the spring of 1933, to compare growth and survival capacities of certain tree species under the varying conditions found on the sand plains, with a view to partial elucidation of the problem of the climax as well as to secure more definite data on the apparent capacity of black oak to invade any or all of the habitats now available. Accordingly, 145 germinating acorns of black oak (from the leaf litter in the oak forest), 43 germinating beech seeds, and 165 2-year hemlocks (from the Yale forest preserve nursery) were planted in various locations on the plains. Some of the plantings were destroyed through fire and cutting. The data in table 6, therefore, are based only upon the plantings which were not disturbed. It has been possible to summarize the data under seven types of habitats, as shown in the table.

It will be seen from table 6 that approximately one-third of the

oaks, one-sixth of the hemlocks, and none of the beeches have grown and persisted for 2 years. Survival of hemlock, as expected, occurred only under partial and full shade, primarily on normal soils. Almost

TABLE 6  
PLANTING, GROWTH, AND SURVIVAL DATA FOR THREE TREE SPECIES

DATE OF PLANTING AND OBSERVATION	HABITAT*							TOTAL OF ALL COMMUNITIES
	1	2	3	4	5	6	7	
<i>Quercus velutina</i> , germinated acorns								
Planted April 27, 1933.....	53	12	38	13	....	10	6	132
Living and visible above ground	6	3	8	3	....	2	0	22
	14	5	11	6	....	3	4	43
	22	7	20	12	....	7	4	72
	10	4	17	13	....	0	1	45
<i>Fagus grandifolia</i> , germinated seeds								
Planted May 1, 1933.....	6	3	3	6	....	6	7	31
Living and visible above ground	3	1	1	0	....	1	0	6
	2	1	1	1	....	4	1	10
	2	0	0	0	....	1	1	4
	0	0	0	0	....	0	0	0
<i>Tsuga canadensis</i> , 2-year seedlings								
Planted May 2, 1933.....	44	3	13	16	4	34	9	123
Living and visible above ground	35	3	12	16	4	34	9	113
	34	3	12	16	4	30	8	107
	30	3	12	16	4	30	6	101
	0	0	5	0	2	10	4	21

\* 1: *Trichostema-Andropogon* community, open; 2: same, partial shade of oaks; 3: same, full shade of oaks; 4: *Andropogon-Cladonia* association, open; 5: same, partial shade of oaks; 6: *Pinus rigida* association, full shade; 7: *Quercus* spp. association, full shade.

complete failure of the oaks in the forests was unexpected, but the data are too scanty to warrant far-reaching conclusions. Naturally-growing seedlings were present on all sides. Perfect survival of the

oaks in the *Andropogon-Cladonia* association may probably be considered significant, especially when contrasted with the relatively poor survival on the three types of habitat in the *Trichostema-Andropogon* community. This differential survival is undoubtedly related to a whole complex of aerial and edaphic factors which have different values in the various habitats, as will be brought out in later sections.

At least forty-one of the fifty-three oaks originally planted to depths of 2 inches in open sand of the *Trichostema-Andropogon* community (table 6) had grown considerably after planting, as shown by excavation in the summer of 1935. Many had developed strong tap roots to depths of 6 to 12 inches before dying late in 1933 or in 1934. A number of these, however, had not sent shoots above ground. Of the twenty-two which were visible on June 13, 1933, fully half could be seen only as small growing tips almost even with the surface. These apparently never developed further. This behavior is undoubtedly comparable with that described on page 234 for the same species in squirrel caches, and indicates again with what difficulty black oak invades the open *Trichostema-Andropogon* community. The ameliorating influence of shade aids growth and survival, as shown in columns 2 and 3 of the table. Therefore the lethal effect is not due solely to chemical conditions of the soil, which must be essentially the same in the two locations, but must also be related to the higher temperature and evaporation rate in the open habitat. More favorable soil conditions in the *Andropogon-Cladonia* association compensate for a similar exposure there, and thus black oak has best chances for survival in that community.

No attempt was made to measure the size of living individuals in 1935. Because of the limited survival, it was felt that averages would have little significance. It should be noted, however, that, just as in squirrel caches, many of the oak seedlings had died back one or more times, and had sprouted again from lateral and cotyledonary buds. Some of them were also partially stripped of their leaves by insects during the summer of 1935. Many of the hemlocks also showed marked evidences of living under conditions of stress. Not too much significance should be attached to present survival values of either species.

## Historical factors in differentiation of the vegetation

### PRE-COLONIAL PERIOD

Since affinities of the sand plains flora are primarily southern it seems reasonable to suggest, as BROMLEY (9) has done for the oak and pitch pine forests of southern New England, that complete establishment of this flora occurred during a postglacial xerothermic period. The influences of such a period, well known in the Middle West (29, 50), might have extended to the Atlantic seaboard. RAUP (48) came to the conclusion that a "warmer and drier climate has occurred in New England within the past 3000 years."

BROMLEY (9) stressed the importance of the custom of the Indians of southern New England in burning forest and grassland. To him it seemed certain that at the time of white settlement "most of southern New England was not typical forest, but a woodland greatly modified by fire and anthropic factors," and that "on many of the higher, overdrained sandy or gravelly knolls were open areas dominated by *Andropogon scoparius*, the climax grassland of such sites in southern New England." On the other hand, RAUP (48) did not attach much importance to fire as a factor in the creation and maintenance of the open woodlands near the coast.

Significant for the sand plains area is a statement by TRUMBULL (55), who spent most of his life, from 1760 to 1820, in the township of North Haven. Although it is made with reference to all of Connecticut, there seems to be no reason for doubting that his intimate contacts, both with the local area and with the immediate descendants of its original settlers, formed a partial basis for the statement that "the Indians so often burned the country, to take deer and other wild game, that in many of the plain dry parts of it, there was but little small timber. Where lands were thus burned there grew bent grass, or as some called it, thatch,<sup>10</sup> two, three and four feet high, according to the strength of the land. This with other combustible matter, which the fields and groves produced, when dry, in the spring and fall, burned with violence and killed all the small trees. The large ones escaped, and generally grew to a notable height and magnitude."

<sup>10</sup> This could well be *Andropogon scoparius*.

TRUMBULL's opinion agrees with a more specific statement by THORPE (54), who wrote, "it was not true then [referring to the period of settlement], as now, that in the Fifth Division" the soil was thin and sterile, for we know as early as 1738 these plains were hunting grounds for deer by the Indians, and that a certain wiry and ill-favored grass grew so dense and tall as to literally conceal from view one walking through it. Here also grew "great oaks and much scattered, a few of which remain to this time." THORPE had access to many old documents belonging to the descendants of the early settlers of North Haven, and there is no reason to suppose that his statements were not based on satisfactory evidence. One may well wonder, however, whether 1738 is not a typographical error for 1638, the date of founding of New Haven Colony, and the time of purchase of all of these lands from the Indians. According to DE-FORREST (18) there were only four Indians left in Wallingford in 1774, and only eleven in East Haven. The last sachem of the Quin-nipiacs died between 1730 and 1740.

THORPE's statement definitely suggests widespread development on the North Haven-Wallingford plains of an original grassland vegetation, in which oaks were scattered. The general picture would thus have resembled, except for the taller and denser growth of the grass at that time and the lack of fence line communities, the present *Andropogon-Cladonia* association. This original grassland cover has previously been postulated by the writer on the basis of the character of the A horizon in the undisturbed soil profiles.

#### COLONIAL PERIOD

SETTLEMENT.—About one hundred persons commenced the settlement of Wallingford in the 1660's (16). The usual method of land allotment (11) was followed, each of the original thirty-eight planters receiving small tracts in various parts of the town, in addition to his house-lot in the village. Although these allotments did not include the plains area, apparently, the number of planters had increased to one hundred and twenty by 1700, and it is probable that

<sup>11</sup> This term refers to the fifth apportionment of lands in New Haven Colony in 1710-11. Most of the North Haven portion of the sand plains areas under consideration was in this division.

the area was put under cultivation shortly thereafter. The southern boundary of the town through the plains was and is the transverse stream shown at the bottom of figure 1 and at the top of figure 2.

The main road from Wallingford to New Haven seemed to follow the same general line as the present one, easily visible as the right of the two major diagonals in figures 1-3. It thus traversed the entire length of the plains. At Wallingford this road connected with roads to Middletown and Hartford, and was the main artery of travel between these points and New Haven until 1798. Along this road passed many travellers from whose diaries quotations about Wallingford and North Haven have been obtained.

THORPE (54) stated that several families settled in the area which is now the village of North Haven (partly visible in the lower left corner of figure 3) between 1670 and 1700, and that the domain of the present township lay principally in the third, fourth, and fifth divisions of New Haven Colony. These apportionments were made in 1680, 1704, and 1710-11 respectively (19). The sand plains area in North Haven was principally in the fifth division. The lands were not all taken up when allotted, however, and it was not until 1767 that all public lands of the colony passed into individual ownership. It is possible that in places the original vegetation was relatively undisturbed until that late date, although the population in the northern part of the North Haven plains had increased to the point that a school was established there in 1763 (54). Its district extended to the Wallingford line.

CULTIVATION.—Records of settlement indicate that by 1750 much of the land in the plains was being utilized. The very small size of the rectangular plots which had been fenced off, now marked by rows of trees in the old fence lines in both barrens and *Andropogon-Cladonia* communities, would indicate cultivation rather than grazing use. Pasture areas, even when not held in common, would have been larger. The largest barren area apparently undivided by such lines is that seen near the northern end of the Wallingford plains (1 in figure 1). This area, however, is actually divided into halves by a nearly continuous low ridge extending across it from east by south to west by north. This ridge must have been formed along a fence line, through deposition of sand.

FENCES.—Fence building was one of the prime duties of the New England colonists, and elaborate regulations were made concerning it (47). The lack of fences was more a subject for comment than their presence, for DWIGHT (20), referring to a plain near Windsor in 1797, wrote that “the fields on this plain, which are considerably numerous, are unenclosed. This species of agricultural economy . . . . in this state exists, I believe, here only.”

Two lines of evidence support the statement made previously that the fields in North Haven and Wallingford were inclosed with zigzag rail fences. This type of fence was uncommon in New England, and called for comment on the part of travellers. GEORGE WASHINGTON (56), travelling from New Haven to Wallingford on October 19, 1789, noted that “these sandy lands afford but ordinary crops of corn . . . . The lands (stone being less) are in part enclosed with posts and rails.” SAMUEL DAVIS, after travelling from Wallingford to New Haven on September 2, 1789, wrote in his journal (17) that “after leaving Keyes’s [in Wallingford] we travelled on a level, sandy plain,—a barren heath of some length. . . . See much Virginia fence this stage. Pass through North Haven, the lightest soil we have yet seen in this state. See many locust-trees, which do not appear injured by the worm. Farmers sowing wheat all along this distance.” Virginia fence was the name commonly applied to zigzag rail fences by New Englanders (1).

More important than historical evidence from journals as to the extensive construction of rail fences on the plains is the direct evidence still available in the field. Although in most cases the rails are completely gone, many of the rounded stones which the settlers brought to use as bases for the fence are still in their original positions. They were set into the A horizon of the soil so that their tops projected slightly above the surface, one at each angle of the fence. Their distances apart, 11 to 12.5 feet following a zigzag course, and 18 to 21 feet in a straight line, indicate the length of the rails and the angles formed. These stones have an important bearing on the present problem.

The importance of these fence rows as centers of refuge for the original vegetation cannot be underestimated. No doubt some of the rare species in the original sand plains flora were eliminated com-



pletely during the period of cultivation. Others, such as *Viola pedata*, have persisted in these rows, but have not been able to spread from them since.

The invasion of bird- and wind-dispersed woody plants into the fence rows is a logical assumption, but its occurrence is also supported by facts in the field, in that they still persist along many of these fence lines, especially those traversing the *Andropogon-Claudia* association. The occurrence of *Nyssa sylvatica* in the rows can be explained logically on no other grounds than that of bird dispersal. *Juniperus virginiana* apparently had a more important role than it seems to have at present. Fire-scarred stubs and stumps of this species have been found repeatedly in these rows, many of them cut long ago. Five such stumps in one row, cut about 1 foot above the ground, showed 81, 88, 91, 101, and 106 growth rings in 1932, and ranged from 11 to 16 inches in diameter at that level. These had been cut for many years, as shown by the degree of decay, a slow process in this species. It seems safe to assume an origin as early as 1800, or before. At present the principal woody growth in this row is *Quercus ilicifolia* and *Prunus serotina*, species capable of continued life through sprouting.

The artificial introduction, into fence rows and elsewhere, of tree species which were not proper constituents of the sand plains flora probably occurred at an early date. Their persistence is better evidence of such introduction than any historical record, although in the middle of the eighteenth century ELIOT (21) referred to buttonwood trees (*Platanus occidentalis*), planted for fence and firewood, and locust (*Robinia pseudoacacia*) and buttonwood planted in pasture land in various places in New England. DAVIS (17), in 1789, had noted the abundance of black locust in North Haven.

#### POST-COLONIAL PERIOD

Effects following the improvement of the sand plains through cultivation and fencing were apparently what one would expect on the basis of present-day knowledge of such areas. They illustrate forcibly the results of the unwise use of land which was, and unfortunately still is, too prevalent among American agriculturists. Depletion of mineral nutrients, brought about through cropping and

cultivation, was rapid in the sandy soils. Although it is probable that many owners employed some or all of the various crude methods of fertilization in use at that time, the crop return soon failed to justify the labor and expense involved, and much of the land was abandoned.

In many of the areas, however, the soil was still suitable for re-establishment of natural or semi-natural vegetation, and it probably spread into them in the manner indicated on pages 246-249. Among the trees which entered, species such as black locust, gray birch, and red cedar were important. Thus in 1795 it was noted that "some silver firs are thinly scattered over this tract, and make but a poor appearance. It has not the appearance of a territory fit for tillage, but may answer well enough for pasture-land" (35). The reference is to the North Haven plain, and the trees mentioned must have been red cedars.

WIND EROSION.—An effect much more harmful to re-establishment of the natural vegetation than soil depletion was the actual soil destruction, which occurred on many of the areas soon after plowing. The loose sand, especially that with low colloidal content, must have been subject to wind erosion almost as soon as the original vegetation was destroyed. While no reference has been found to wind erosion in cultivated fields in the towns of Wallingford and North Haven, the process was not unknown in Connecticut at an early date. DWIGHT (20) in 1800 wrote that "we rode out . . . . to see a field, from which the surface had been blown away, in a manner uncommon to this country, and supposed by the inhabitants to be singular. A discreet man, who lived in the neighborhood, informed us, that, about forty years since, the soil, on a little spot in the interior of the field, was blown off; and that the sand, which lay beneath it, and which was very fine and light, was then blown out, so as to form a small cavity. Hence the ravage extended in every direction, until about twenty acres were uncovered to a depth of from one to three feet." A similar process undoubtedly occurred in fields of North Haven and Wallingford. Thus we may account for DAVIS' reference (17) to a "level, sandy plain—a barren heath of some length,—" in 1789. In 1797, DWIGHT (20) wrote that "North Haven lies on both sides of Wallingford River; and comprises the

valley and a part of the bordering hills. The soil of the valley is partly rich interval land, and more extensively sand, covered with a thin stratum of loam; light, but warm; and yielding tolerable crops of rye. Near the northern limit of the township it is so light, as in two or three places, of small extent, to be blown into drifts. In these places it is absolutely barren."

The sand drifts which DWIGHT mentioned undoubtedly were wind deposits derived from the A horizon of the cultivated fields. As wind erosion proceeded, this horizon was completely removed, and the B horizon was exposed. Erosion was probably fairly rapid at first, but later slowed down with the partial formation of a desert pavement from the gravel particles mixed with the sand. Nevertheless a bare habitat had thus been created which was much more rigorous in its selective action upon immigrants than was the ordinary abandoned cultivated field which was not eroded, and the way was paved for the *Trichostema-Andropogon* community still present on such areas. Dynamic tendencies within this community have already been discussed.

**SAND DEPOSITION.**--The wind-borne and wind-sorted sand was deposited chiefly in and along the south and east fence rows of the field from which it was derived. In the course of a few decades it had accumulated to considerable depths, especially along boundaries of the larger fields, thus forming the substratum for the *Polygonella-Quercus* community. The fence rails were probably removed soon after abandonment, for wood was becoming a valuable commodity in many Connecticut towns by 1800. Shrubs and trees which had invaded the fence rows, and the original vegetation which had persisted in them, served to catch the drifting sand. Probably many of the trees and shrubs survived the burial of their basal parts, and some have persisted to this day. The stones which formed the bases for the rail fences were buried in the sand ridges. These stones, still imbedded in the A horizon of the old soil profile, have been uncovered by excavating to the bottoms of three of the wind deposits. This seems to be adequate proof of the method of origin of sand ridges and barrens here proposed.

That the sand deposition was and is a continuative process has been suggested (p. 242). This was vividly shown by the partial exca-

vation of the stumps of a number of oak trees which had been cut during the winter of 1934-35. These trees grew on and near the crest of a sand ridge which extends along the south side of the barren area at 2 in figure 2. Eighteen stumps ranged from 10.5 to 22 inches in diameter at the cutting level, and showed from forty-three to seventy-eight growth rings. A number of them were in groups of two or three, every individual in a group showing approximately the same number of rings. This would indicate origin in squirrel caches. There seems to be a general tendency for this dune, which is now 55 feet wide, to shift southward. Its present crest extends in a generally straight east and west line 8 to 10 feet south of the center line of the old fence row, while locally wind sweeps have built up crests even farther to the south. Therefore a tree on the north side of the dune may have its large uppermost lateral roots exposed near the base, while one on the south side often shows a deeply buried root collar. The depth of this collar below the present surface of the dune and its height above the old A horizon, correlated with the age of the tree as shown by ring counts, give some clue as to rate of sand movement and deposition, both before and after germination of the acorn. Two trees, located near the crest of the dune but 18 feet south of the center line of the old fence row, had been buried by 21 inches of wind-borne sand since germination. The fifty-nine growth rings of the stumps therefore indicate average deposition of at least 1 inch of sand around them every 3 years. These trees had sent branches from their lateral roots upward into the newer deposits. The total depth from the present surface to the old A horizon was 44 inches, indicating that it has been approximately 120 years since the blowing sand first buried the A horizon at this particular spot. The old profile was well developed, the A and B horizons having thicknesses of 8 and 22 inches respectively. Another stump, showing fifty-seven growth rings, was located at the exact center of the dune, which here coincided with its crest, 50 feet to the east of those just described. The depths to the root collar and to the tops of the A, B, and C horizons of the old soil profile were 16, 46, 53, and 77 inches respectively. Using the rate of deposition indicated during the past 57 years, the age of the dune at this point would be about 180 years. Another stump, located at the crest of the dune and 10 feet to the

northwest of the stumps first described, showed sixty-nine growth rings 48 inches above its root collar, approximately breast height. Depths from the present surface to root collar, and to the A, B, and C horizons of the old soil profile, were 31, 45, 54, and 76 inches respectively. The extension of a scar and callus, 23 years old, to a depth of 14 inches below the present surface, suggests rapid recent deposition. The average deposition prior to the injury would not have been more than 1 inch every 3 years, as shown by an indicated age of over 75 years for the tree. Applying this rate to the earlier deposits, one arrives at an approximate age of 120 years for the dune, at this location. It seems safe to assume that the origin of this dune therefore dates back to 1800 or earlier, for these stumps were all located to the south of the old fence row. In its early stages, it must have been one of the sand drifts seen by DWIGHT in 1797, near the northern border of North Haven.

ABANDONMENT.—An examination of the tax lists of North Haven<sup>12</sup> for the 30 years following the incorporation of the township in 1786 revealed a decrease in cultivated land during the period. In 1787, taxes were paid on 2,105 acres of "plowland" and on 2,105½ acres of "bush pasture." In 1816, these figures stood at 1,496 acres of plowland and 4,119¾ acres of bush pasture. It is logical to suppose that these changes were in part the result of abandonment of cultivated land on the plains. Further indications of the almost complete abandonment of the North Haven sand plains area in the first half of the nineteenth century are the statements of THORPE (54) that in 1841 the fifth school district (located on the northern part of the North Haven plains) had become so reduced in numbers that the school was finally abandoned. THORPE wrote in 1892 that the schoolhouse and thirteen old homes in this district had disappeared.

Abandonment probably occurred sooner in Wallingford than in North Haven, for it was the older community. No data have been obtained which indicate the exact time, but it had proceeded to such an extent by 1819 that PEASE and NILES (46) were able to write as follows: "Wallingford plain, situated upon the eastern bank of the

<sup>12</sup> I am indebted to Mr. BLAKESLEE, town clerk of North Haven, for permission to examine these records.

Quinipiack, is a very singular tract of land. It . . . is the most extensive tract of level land in the state; and, under its present cultivation, the most sterile and barren. Its soil is a coarse sand; and it seems to be considered so barren as not to be worth cultivation, a considerable proportion of it being wholly unenclosed. Yet there is but a very small proportion of it which blows, or but what has sufficient consistence of soil, or the upper surface of the land, to sustain itself, and to retain the vegetable substances, and other manures which collect, or are deposited upon it. Notwithstanding the sterile appearance of this land, it is believed, that by a judicious and ameliorating system of cultivation; by the use of clover, sheep, and summer fallow; or by the application of some earths or manures, calculated to correct the predominating silicious character of the soil; it might be rescued from its present condition, a waste and agricultural void, and rendered suitable and valuable for a grain culture." Apparently no serious attempts were made to follow the advice given in this quotation.

Portions of the area soon passed into the ownership of the railroad company, which built the line that now traverses the plains, in 1839. Aside from the sand pits which were dug in the northern part of North Haven (4 in figure 2), the company's land has since been subjected to relatively few anthropic influences, except that of fire and of tracking. The same has been true of much of the land owned by individuals. Aside from the destructive effects of frequent fires, especially on the woody species, and occasional cutting of the latter, the vegetation of many areas has been free to develop without serious anthropic influence during the past 100 years. The types of vegetation which are now found may therefore be correlated in large part with the difficulty or ease with which the various species could become established under the physical and chemical conditions prevailing in the normal, truncated, and buried soil profiles of the various areas. Reference to the section on vegetation and to figure 5 will reveal the approximate method, rate, and degree of successional change which have followed the creation of each of these three initial bare areas.

### Continuative factors in differentiation of the vegetation

Some of the factors which show more or less marked differences among the present plant communities and habitats have been measured, and the data are now presented. No attempt is made to evaluate the significance of individual differences upon the ecesis of plants in the various habitats. That the resultant of the complex of factors acting within the *Trichostema-Andropogon* community is most unfavorable for plant invasion has been suggested in the previous discussion. This becomes obvious when one bears in mind the multiplicity of factors affected by complete removal of the A horizon of a soil developed in a substratum of sand. The effect of such removal in hindering invasion is well demonstrated by the paucity of vegetation on areas of this type which are more than 125 years old. Experimental work which has been performed thus far upon the species dealt with, however, is not sufficient to allow a close analysis of the significance of the differences of individual factors upon ecesis in any particular case, and in large part the facts must therefore speak for themselves. The data are also incomplete in that no analyses have been made of available nutrients. The time available did not permit detailed analyses with the refined technique essential to the establishment of significant differences in soils in which even the most favorable show very small amounts of such nutrients at all times. Such variations are masked through the experimental error in rough techniques. That such differences occur, especially in available nitrogen, between the A horizon of a normal profile and the top layer of a truncated profile, must be assumed.

Stations were maintained at which an attempt was made to measure a number of factors periodically during the summer of 1932, but it proved impossible to carry through this program as completely as planned. It was realized too that in a problem involving only a small area within a single climatic region, measurement of factors during a time of maximum stress, and comparison of the values obtained in the different communities, would give results of much greater significance in proportion to the labor involved than would accumulation of the data necessary for a seasonal average. One cannot overemphasize the importance of the extreme or unusual value as a factor in the local or general distribution of plants. In the

presentation to follow, therefore, even when a series of values has been available, data have been selected so that the extremes are emphasized in a contrast of the values among the various habitats.

#### EVAPORATION

Even though the evaporation curve of any type of atmometer is not in exact agreement with the transpiration curve of a plant growing in the same place, it is generally recognized that evaporation values are important indices of habitat, and that they give some idea of the stresses to which plants are subject in various habitats. Evaporation was measured in the *Trichostema-Andropogon* community; in the *Andropogon-Cladonia* association, both in the open and at the bases of the oaks shown in figure 21; and in the pine-oak transition plot for which quadrat data are given in table 5, for periods of 6, 5, 1, and 6 weeks respectively. Standardized spherical Livingston atmometers, fitted with Musch rain-correcting mountings, were used. Two white-bulb and two black-bulb instruments were placed at each station, bulbs being displayed at a height of 20 cm. above the ground. While each datum given in table 7 is thus the average of two measurements, there was almost perfect agreement in each pair. The instruments were refilled every two or three days, but the standardized data have been grouped and computed for the most part as average loss in cubic centimeters per day per week. To afford more ready comparison they have also been computed in percentage loss based on a value of 100 for each type of instrument in the *Trichostema-Andropogon* community.

The data in table 7 show that evaporation rates are lower in the *Andropogon-Cladonia* association than in the *Trichostema-Andropogon* community, differences being more pronounced for white- than for black-bulb readings. This result would be expected because of the influence of the first named community in decreasing wind movement, and in increasing air humidity through transpiration, while solar radiation values should be nearly the same. Since the differences are more pronounced during times of maximum stress, as shown by the data for July 20-22, they may be significant in their effect upon ecesis of plants within the two communities. The fluctuating value of the difference percentage in the *Andropogon-*



*Cladonia* association, based on the constant value of 100 per cent for the *Trichostema-Andropogon* community, may probably be as-

TABLE 7  
EVAPORATION DATA, SUMMER, 1932

PERIOD OF OBSERVATION	TRICHOSTEMA- ANDROPOGON			ANDROPOGON- CLADONIA			PINE-OAK TRANSITION		
	W*	B	D	W	B	D	W	B	D
Average loss in cc. per day per week									
July 18-25.....	40.3	53.8	13.5	35.1	48.3	13.2	17.0	18.6	1.6
July 25-Aug. 1...	28.4	39.5	11.1	26.1	37.8	11.7	12.1	13.5	1.4
Aug. 1-15.....	27.8	37.7	9.9	25.2	35.3	10.1	11.1	12.6	1.5
Aug. 15-22.....	25.5	33.8	8.3	24.1	33.5	9.4	10.7	12.2	1.5
Under oak trees									
Aug. 22-29.....	30.6	43.2	12.6	24.3	30.0	5.7	12.8	14.4	1.6
Percentage loss based on instruments in Trichostema-Andropogon community									
July 18-25.....	100	100	100	87.2	89.8	97.6	42.2	34.7	12.2
July 25-Aug. 1...	100	100	100	91.9	95.8	105.7	42.6	34.3	12.9
Aug. 1-15.....	100	100	100	90.6	93.5	101.4	40.1	33.5	15.0
Aug. 15-22.....	100	100	100	94.4	99.3	114.6	42.1	36.2	18.0
Under oak trees									
Aug. 22-29.....	100	100	100	79.5	69.4	45.1	41.9	33.5	13.0
Extreme average loss in cc. per day									
July 20-22.....	58.9	74.7	15.8	49.5	63.5	14.0	26.2	28.5	2.3

\* W, white bulb; B, black bulb; D, difference.

cribed to differences in re-radiation within the two communities, under changing conditions of soil moisture, air humidity, cloudiness, etc.

Within the pine-oak transition, the black bulbs gave readings which were relatively lower in comparison with those in the *Trichostema-Andropogon* community than did the white bulbs. This would also be expected, since the difference between the two readings is caused primarily through the much greater absorption of solar radiation by the former, and is considered to be a rough measure of that factor. Thus for the week of August 22-29, the percentage value of incident radiation at the level of the atmometer bulbs was 100, 45.1, and 13.0 in the *Trichostema-Andropogon* community, under oaks in the *Andropogon-Cladonia* association, and in the pine-oak transition region respectively. This indicates approximately the relative amount of shade afforded by the isolated oaks in contrast to that of the forest.

Total evaporation from the white bulbs in the *Trichostema-Andropogon* community and the pine-oak transition during the 6 weeks' period from July 18 to August 29, 1932, was 1263 and 527 cc. respectively. The figures are in striking contrast with those recorded by BALDWIN (2) during the same period at Long Lake, Hamilton County, New York. BALDWIN's instruments were exposed at a 6 inch level in an opening within and under the complete canopy of a paper birch-white pine-balsam fir forest. The values recorded in the two habitats for the 6 weeks' period total 172 and 471 cc. respectively. The total evaporation from white bulbs at the open station in the Adirondack Mountains was less than that in almost complete shade in the pine-oak transition forest of the North Haven sand plains. However, highest readings on the Connecticut sand plains in the *Trichostema-Andropogon* community are low in comparison with evaporational stresses to which *Andropogon scoparius* is exposed in some parts of its range. Thus, during the severe drought of 1934, the average daily evaporation by weeks in the prairie at Lincoln, Nebraska, was continually over 40 cc. per day, and ranged as high as 80 cc. from May 15 to September 1 (58). Failure of this species to spread more rapidly over the barren areas must probably be attributed more to other factors than to excessive evaporation, although this factor undoubtedly is significant in the lack of ecesis of some plains species on these areas.

## SOIL TEMPERATURE

The importance of high soil temperature, especially at the surface, as a factor in the failure of cecis has been demonstrated for a number of tree species by various workers. Temperatures between 120° and 130° F. may be regarded as critical for many species. MILLER (40) states that "the thermal death point of most plant cells lies between 45 and 55 degrees C., and that, in many cases at least, the fatal temperature must be prolonged some little time before death ensues."

In view of the high temperatures which have been recorded for sandy soils, it was thought advisable to obtain data on the maximum

TABLE 8

MAXIMUM SOIL TEMPERATURE, DEGREES FAHRENHEIT, SUMMER, 1932

DATE	TRICHOSTEMA- ANDROPOGON		ANDROPOGON- CLADONIA		OAK-PINE TRANSITION	
	SURFACE	4 IN.	SURFACE	4 IN.	SURFACE	4 IN.
June 27.....	133.0	.....	116.0	.....	93.0	.....
June 29.....	143.0	95.0	133.5	88.0	85.5	67.0
July 7.....	133.0	89.0	120.0	85.0	87.0	67.0
July 14.....	147.0	94.0	142.0	90.5	88.5	69.0
July 16.....	145.0	94.0	141.0	93.0	95.0	67.0
August 1.....	119.0	89.5	116.0	88.0	78.0	65.0
August 6.....	126.0	95.0	120.5	89.5	79.5	70.5
August 24.....	127.0	91.0	120.0	87.0	74.0	67.5

surface soil temperatures in various communities in the sand plains. These were measured with maximum thermometers of the gooseneck type. Temperatures at depths of 4 inches were also obtained with standard maximum thermometers at the same stations during the summer of 1932. The records given in table 8 have been selected to emphasize the extremes.

The data show that the maximum temperatures of both surface soil and that at a 4 inch depth reach higher values in the *Trichostema-Andropogon* community than they do in the *Andropogon-Cladonia* association. Whether the differences are great enough to be significant is open to question. During periods of stress the temperatures are high in both communities and the differences are

lessened. Little rain had fallen during the two weeks preceding the measurements of July 14 and 16, so that the surface soil in both communities was thoroughly dried out, and had a low specific heat, with consequent high maximum temperatures. That differences between the values in these two communities and in the pine-oak transition would be significant in the ecesis of mesic plants needs no further emphasis.

During the summer of 1935, a study was made of the effect of the shade of an isolated oak in the *Trichostema-Andropogon* community upon the maximum surface soil temperatures under and outside the

TABLE 9

MAXIMUM SURFACE SOIL TEMPERATURE, UNDER AND NEAR ISOLATED OAK  
IN TRICHOSTEMA-ANDROPOGON COMMUNITY, SUMMER, 1932

DISTANCE AND DIRECTION FROM TRUNK	JULY 1	JULY 3	JULY 5	JULY 11	JULY 12
21 ft. south			146 5	150 0	148 5
17 ft. south	144 0	142.0	149 0	152.5	154 0
9 ft. south	147 0				
2 ft. south	95 5	102 5	99 0	97 0	105.0
0 ft. south	87.0	84.0	89 5	85 5	89 0
2 ft. north		83.0	92 5	88 0	92 5
4 ft. north	94 5				
6 ft. north	81.5	80.0	90.0		
8 ft. north	125 5	122 5	128.0	118 5	123.0

influence of its crown. Gooseneck maximum thermometers were placed at intervals along a north-south line passing through the base of the tree. The data in table 9 illustrate strikingly the much more favorable condition for ecesis under the isolated oaks than in the completely open portions of the *Trichostema-Andropogon* community, at least so far as maximum surface soil temperatures are concerned. This difference in temperature is of great importance in influencing the relative rate of drying of the surface soil in the two habitats. Although no natural vegetation occurred under this particular tree, fourteen of the twenty-four black oaks and four of the eight hemlocks which were planted under it in 1933 were still living in 1935, in contrast to the complete failure of eighteen hemlocks and the persistence of only two out of seventeen oaks planted in the open.

Practically simultaneous temperature measurement at various depths of the soil profile in the *Trichostema-Andropogon* community at 1 P.M. showed an interesting curve. The character of the decrease may be briefly illustrated by the data for July 11, 1935. With a surface soil temperature of 145.5° F., temperatures of 133.0°, 113.0°, 94.0°, 84.0°, 79.5°, and 78.0° were found at depths of 1, 2, 6, 12, 20, and 31 inches respectively. It is not surprising that the surface 2 inches of soil is constantly dry in this community except during and immediately after periods of precipitation, and that roots at depths of 6 to 12 inches show very rapid growth.

#### AVAILABLE SOIL MOISTURE

Measurement of this factor is of importance in regions lacking uniformity in seasonal distribution of precipitation. In areas with regular and heavy rains it is of less value, for the water content in the soils of many communities in such environments is always considerably above the permanent wilting point, as shown by usual methods of determination. Under such conditions this factor is not usually considered to be of much significance, except when in excess. Therefore, although determinations of available water content were made weekly in various stations for a period of 10 weeks during the summer of 1932, for the most part only those data are presented in which there seems to be a significant difference in the amounts of available water among the various communities.

Soil samples in duplicate were secured in borings 2 feet apart with a sleeve-type augur at the depths indicated in table 10. Each datum thus represents an average of two determinations. The samples were placed in metal boxes, and, after weighing, were dried to constant weight at 105°-110° C. Water content values were computed on the oven-dry basis. Moisture equivalent values were determined on air-dried portions of the samples by means of a moisture-equivalent centrifuge, and were converted to wilting coefficients as recommended by BRIGGS and SHANTZ (7). Available water was calculated in the usual manner. The water content data were also converted to relative wetness values, according to the method of CONRAD and VEIHMEYER (15). In this determination total moisture content is divided by moisture equivalent of the same sample, and the quo-

tient is multiplied by 100. The assumption is made that the moisture equivalent is a reasonable measure of the maximum field capacity of a soil. Therefore a relative wetness value of 100 indicates that the soil is 100 per cent wet; one of 54.35 is at the wilting coefficient. While use of the factor 1.84 in computing wilting coefficients from moisture equivalents, and the assumption that moisture equivalent is equal to field capacity, are both open to serious question, the results obtained give a better idea of the total water relationships in the soil than do those of available water alone. Both values are presented in table 10.

The data show that even under conditions of fairly severe drought the amount of available soil moisture in the *Trichostema-Andropogon* community and in the *Andropogon-Cladonia* association is always considerably above the wilting coefficient, except in the surface 6 inches. The low values at this level are caused by the effect of the completely dry surface 2 inches of soil in lowering the average. Below this depth the sand was always moist. It would therefore seem that lack of soil moisture is not a factor which inhibits invasion of many perennial plants into these areas. During a number of years, periods occur in which the surface 2 inches of sand is sufficiently wet for a long enough time to enable such plants to germinate, and send their roots to the permanent water supply located relatively near the surface. In any one year, however, the abundance of annual plants is undoubtedly related to the length and number of such periods during the critical period of germination and establishment.

In the sweet fern, pine, and oak communities, water apparently does become almost or completely unavailable at varying depths during periods of drought, as shown by the data for July 20, 27, August 10, and 17. Less than half an inch of rain fell in the 3 weeks' period from July 2 to 22, and evaporation rates were high. Consequently transpiration of the denser types of vegetation caused a withdrawal of most of the available water from the soil. The 2.5 inches of rain which fell between July 22 and August 10 did not suffice to rewet the soil in the pine woods beyond a depth of 2 feet, and the situation remained the same on August 17, even though another inch of rain fell during the intervening week. It would seem,

TABLE 10  
AVAILABLE WATER AND RELATIVE WETNESS IN VARIOUS  
STATIONS, SUMMER, 1932

DEPTH (INCHES)	T-A*		A-C		Ma		Pr		Qs	
	AW	RW	AW	RW	AW	RW	AW	RW	AW	RW
June 20										
0-6.....	0.9	71	3.9	113	.....	.....	33.1	364	.....	.....
6-12.....	3.3	121	5.7	136	.....	.....	5.7	144	.....	.....
12-18.....	4.4	148	6.5	142	.....	.....	5.6	151	.....	.....
18-24.....	4.3	168	6.7	155	.....	.....	4.7	139	.....	.....
24-30.....	4.3	187	6.4	150	.....	.....	4.6	140	.....	.....
30-36.....	3.7	207	5.5	153	.....	.....	3.9	141	.....	.....
July 12										
0-6.....	0.0	54	0.5	61	.....	.....	1.6	69	.....	.....
6-12.....	3.1	118	4.5	119	.....	.....	1.5	78	.....	.....
12-18.....	2.9	116	4.9	121	.....	.....	2.2	92	.....	.....
18-24.....	2.5	120	4.9	129	.....	.....	1.8	87	.....	.....
24-30.....	3.2	153	5.2	132	.....	.....	1.5	83	.....	.....
30-36.....	3.1	182	4.1	129	.....	.....	1.1	78	.....	.....
July 20										
0-6.....	— .1	52	.....	.....	.....	.....	0.5	59	1.9	81
6-12.....	2.7	108	.....	.....	.....	.....	0.7	65	— .1	53
12-18.....	2.9	116	.....	.....	.....	.....	0.8	69	0.1	57
18-24.....	2.1	111	.....	.....	.....	.....	0.4	61	— .1	53
24-30.....	2.9	144	.....	.....	.....	.....	0.2	59	0.5	64
30-36.....	2.7	105	.....	.....	.....	.....	0.6	67	0.3	61
July 27										
0-6.....	5.9	172	8.6	184	7.3	151	.....	.....	.....	.....
6-12.....	6.6	188	4.8	124	5.1	141	.....	.....	.....	.....
12-18.....	4.9	159	5.2	127	1.6	82	.....	.....	.....	.....
18-24.....	2.7	125	4.2	117	0.7	69	.....	.....	.....	.....
24-30.....	3.1	150	4.2	117	— .1	53	.....	.....	.....	.....
30-36.....	2.7	105	3.4	116	0.1	57	.....	.....	.....	.....

\* T-A, *Trichostema-Andropogon* community; A-C, *Andropogon-Cladonia* association; Ma, clump of *Myrica asplenifolia*; Pr, *Pinus rigida* association; Qs, *Quercus* spp. association. AW, available water; RW, relative wetness.

TABLE 10—*Continued*

DEPTH (INCHES)	T-A*		A-C		Ma		Pr		Qs	
	AW	RW	AW	RW	AW	RW	AW	RW	AW	RW
August 10										
0-6.....	0 7	68	1 9	83	.....	.....	5.7	108	— .4	49
6-12.....	3 6	127	5.2	120	.....	.....	1.0	70	0 6	64
12-18.....	3 3	125	3 1	97	.....	.....	1.3	76	0.5	65
18-24.....	3.0	133	4 2	118	.....	.....	1.4	80	0.5	65
24-30.....	3.1	148	4 0	114	.....	.....	0.2	58	0.3	60
30-36.....	3 2	186	3.3	113	.....	.....	0.4	63	0 4	63
August 17										
0-6.....	— .1	52	0 1	55	0.4	60	14.0	104	.....	.....
6-12.....	3.5	126	4.7	122	2.1	89	1.8	82	.....	.....
12-18.....	2.9	116	4.5	116	1.6	82	1.9	87	.....	.....
18-24.....	2.5	120	4.8	127	1.9	95	1.4	80	.....	.....
24-30.....	2.5	131	4 6	123	0 3	59	0.2	59	.....	.....
30-36.....	3.2	188	3.8	122	0.1	56	0.1	56	.....	.....

therefore, that some of the lower soil layers remain more or less permanently dry during the hot summer months. The data for August 10 indicate reduction of soil moisture almost to the wilting coefficient throughout the total depth sampled in the oak forest. The abundant natural reproduction of black oak in both pine and oak forests indicates that lack of available soil moisture is not primarily responsible for the relatively slow invasion of the species into the pioneer communities in which abundant water supplies are always available a short distance below the surface.

The data for June 20 indicate high relative wetness in all three stations, and except in the surface 6 inches, this value is probably actually that of the maximum field capacity at the various depths. Over 2.5 inches of rain fell between May 26 and June 20, and the weather was cloudy much of the time. This shows, since the relative wetness values are around 140 to 150, that the moisture equivalent is not equivalent to the maximum field capacity of these soils.



## SOIL PROFILE STUDIES

It is impossible to estimate the number of times that examination was made of the soil profile in various places. In addition to the many borings and small excavations which were made to secure samples for moisture determinations, and to check correlation of the indicator annual plants with the three principal types of profile, the profile was studied in a number of large soil pits and trenches to a depth of 8 or 9 feet, from twelve of which samples were secured for laboratory studies. In addition, samples for the determination of moisture equivalent and loss on ignition were secured from more than fifty augur borings in other locations. This great number of examinations formed the basis for the description of the typical profile given on page 214, and for the opinion that almost all of the profiles may be referred to that type, or to truncated or buried variations of it. Since the three variants of the profile are not easily comparable as to horizons, data for a particular soil property can be compared in the same table only with difficulty. Neither has it been possible to average many of the data from any except the normal profiles for the same reason. In view of this fact, and in view of the extensive field examination, I feel justified in selecting certain profiles as typical of their kind, and in presenting only a portion of the data from laboratory studies. These have been arranged in two types of tables. In tables 11 and 13, data for single profiles are given, while in table 12 the data from a number of profiles sampled at arbitrary depths have been averaged.

MECHANICAL ANALYSES.—The texture of a soil, so long as it is of a class which will furnish anchorage and is permeable to roots, is of little significance as a direct factor in the growth and survival of plants. Its importance as an indirect factor cannot be overestimated, however, for it has far-reaching effects upon those water and nutrient relations which are direct factors. In the early stages of the present investigation, before the fact was established that nearly all of the vegetation represented subseral stages following cultivation, it was felt that a coarse soil texture had probably been responsible for the persistence of the barren areas, while closed plant communities were developed on sands of finer texture. Since the substratum is composed of stratified drift, one would expect local

TABLE 11

SOIL ANALYSES, INDIVIDUAL PROFILES IN VARIOUS COMMUNITIES

DEPTH (INCHES)	H*	MECHANICAL ANALYSIS						pH	ME	LI
		2-1 MM.	1-0.5 MM.	0.5- 0.25 MM.	0.25- 0.05 MM.	TOTAL SANDS	TC			
Polygonella-Quercus community										
0-1. ....	A	27.84	29.96	18.86	17.29	93.95	3.72	4.4	3.89	1.80
1-4. ....		37.04	27.64	16.38	14.89	95.95	3.22	4.5	3.06	1.00
4-9. ....		18.71	36.15	23.65	14.96	93.47	4.44	4.5	6.47	2.15
9-20. ....		B	21.22	33.68	26.88	12.09	93.87	3.80	4.6	5.97
20-32. ....	B	17.50	31.34	32.73	13.21	94.78	3.58	4.6	5.07	1.15
32-44. ....	C	33.15	37.06	17.73	8.81	96.75	1.95	5.3	2.60	0.80
44-70. ....	C	34.40	42.94	17.60	3.73	98.67	1.12	5.3	1.62	0.55
Trichostema-Andropogon community										
0-2. ....	B	16.38	34.97	26.17	16.91	94.43	3.27	4.4	3.67	1.45
2-10. ....	B	11.27	29.10	31.91	21.25	93.53	3.01	4.5	3.91	1.00
10-12. ....	B	19.73	33.30	25.17	17.75	95.95	2.55	4.5	3.28	0.85
12-18. ....	C	30.55	40.13	18.03	8.32	97.03	1.76	4.7	2.03	0.65
18-30. ....	C	43.17	39.68	10.21	4.68	97.74	1.51	5.2	1.65	0.70
30-51. ....	C	7.23	52.06	30.92	7.99	98.20	1.30	5.2	1.06	0.45
Trichostema-Andropogon community										
0-1.5. ....	B	9.46	19.20	30.42	31.79	90.87	5.04	4.4	5.73	1.85
1.5-5. ....	B	5.89	20.67	36.53	28.21	91.30	4.79	4.4	5.06	1.35
5-13. ....	B	9.55	24.05	36.18	23.46	93.24	4.04	4.5	4.65	1.15
13-24. ....	C	2.35	24.65	50.94	19.27	97.21	1.33	4.9	2.24	0.60
24-36. ....	C	3.13	27.84	38.10	28.71	97.78	1.19	5.2	2.00	0.50
36-50. ....	C	0.77	23.59	46.40	27.36	98.12	1.15	5.2	1.64	0.55
Andropogon-Cladonia association										
0-1.5. ....	A	5.14	21.34	37.92	26.27	90.67	4.69	4.4	8.49	4.80
1.5-8. ....	A	6.62	22.91	36.01	23.56	89.10	6.15	4.5	7.67	2.80
8-14. ....	B	6.74	23.67	37.70	23.06	91.17	4.37	4.7	6.69	1.65
14-29. ....	B	7.53	21.40	38.00	23.80	90.73	4.33	4.9	6.04	1.60
29-42. ....	C	11.59	27.71	40.25	17.18	96.73	1.80	5.3	2.79	0.85
42-66. ....	C	10.93	38.14	35.52	12.54	97.23	1.55	5.2	1.68	0.55

\* H, horizon; TC, "total colloids"; ME, moisture equivalent; LI, loss on ignition.

TABLE 11—Continued

DEPTH (INCHES)	H*	MECHANICAL ANALYSIS						pH	ME	LI
		2-1 MM.	1-0.5 MM.	0.5- 0.25 MM.	0.25- 0.05 MM.	TOTAL SANDS	TC			
Pinus rigida association										
0-1.....	F	.....	.....	.....	.....	.....	.....	3.9	.....	.....
1-1.5.....	H	.....	.....	.....	.....	.....	.....	3.8	.....	.....
1.5-2.....	A	.....	.....	.....	.....	.....	.....	4.0	.....	.....
2-8.....	A	6.94	27.80	29.70	25.80	90.24	5.72	4.5	6.79	2.30
8-13.....	B	8.47	28.82	26.84	26.79	90.92	5.30	4.6	6.03	1.55
13-24.....	B	9.03	25.64	29.65	28.28	92.60	4.40	4.5	5.50	1.45
24-29.....	C	6.59	26.14	34.53	27.84	95.10	2.65	4.6	3.29	1.00
29-42.....	C	13.62	32.93	33.22	16.33	96.10	1.90	4.6	2.19	0.70
42-57.....	C	27.76	41.21	17.57	10.31	96.85	1.57	4.7	1.49	0.60
Quercus spp. association										
0-1.....	F	.....	.....	.....	.....	.....	.....	4.3	.....	.....
1-2.....	H	.....	.....	.....	.....	.....	.....	3.8	.....	.....
2-8.....	A	14.72	33.64	22.03	18.11	88.50	5.25	4.5	9.27	3.25
8-14.....	B	17.10	34.18	22.18	16.82	90.28	4.22	4.7	6.71	1.95
14-20.....	B	18.99	35.32	19.94	18.78	93.03	2.72	4.7	5.47	1.65
20-42.....	C	20.57	33.79	24.60	16.54	95.50	1.75	5.0	2.71	0.70
42-66.....	C	25.47	44.67	12.25	14.17	96.56	1.19	5.1	2.12	0.70

variations in soil texture. With the realization of the subseral condition of the vegetation, it also seemed advisable to see whether some correlation existed between the local soil texture and the degree to which the soils had been eroded by wind, with the consequent effects upon re-establishment of the natural vegetation.

Samples were collected from the full horizon thicknesses of representative profiles. These were air-dried and passed through a 2 mm. sieve. With very few exceptions, the value of the coarse fractions did not exceed 10 per cent, and in most cases was under 5 per cent of the air-dry weight. Through the presence of the desert pavement, the value of this fraction in the surface layer of the profiles from the *Trichostema-Andropogon* community was always slightly higher than that of the layer immediately beneath. Occasionally

TABLE 12

SOIL ANALYSES; AVERAGED DATA FROM SAMPLES AT ARBITRARY  
DEPTHS IN VARIOUS PLANT COMMUNITIES

DEPTH (INCHES)	1		2		3		4		5		6	
	ME*	LI	ME	LI	ME	LI	ME	LI	ME	LI	ME	LI
0-3.....	5.26	2.00	5.26	2.25	12.36	4.95	9.72	5.30	13.58	5.52	19.06	9.90
3-6.....	5.72	1.80	6.24	1.80	10.19	3.15	8.06	2.75	10.14	2.70	8.12	2.70
6-9.....	5.86	1.70	6.24	1.60	9.60	2.60	8.02	2.36	10.11	2.30	7.88	2.30
9-12.....	5.62	1.60	5.84	1.50	9.76	2.39	7.73	2.00	9.92	2.00	8.01	2.10
12-18....	5.49	1.65	5.64	1.40	9.52	2.12	7.25	1.84	9.65	1.95	7.66	1.85
18-24....	3.75	1.15	5.96	1.50	8.77	1.78	6.98	1.76	9.20	1.75	7.53	1.80
24-30....	2.71	1.00	4.54	1.20	8.31	1.40	6.73	1.61	8.08	1.57	6.53	1.60
30-36....	2.47	0.90	2.73	0.85	5.66	1.15	3.70	1.12	3.44	0.90	3.70	1.10

1: *Trichostema-Andropogon* community, 5 profiles; 2: *Hypericum* community, 2 profiles; 3: *Andropogon-Cladonia* association with *Andropogon* cover 30-50%, 6 profiles; 4: *Andropogon-Cladonia* association with *Andropogon* cover 20-30%, 8 profiles; 5: *Myrica asplenifolia* community, 5 profiles; 6: *Pinus rigida* association, 6 profiles.

\* ME, moisture equivalent; LI, loss on ignition.

TABLE 13

SOIL ANALYSES; THREE PROFILES, POLYGONELLA-QUERCUS COMMUNITY

H	DEPTH (IN.)	ME*	LI	DEPTH (IN.)	ME	LI	DEPTH (IN.)	ME	LI	pH
	0-3	3.94	2.7	0- $\frac{1}{2}$	2.52	1.1	0-1 $\frac{1}{2}$	2.80	1.7	4.3
	3-24	2.82	1.1	$\frac{1}{2}$ -12	2.48	0.9	1 $\frac{1}{2}$ -14	2.24	1.1	5.4
	24-45	5.11	2.1	12-30	3.41	1.2	14-16	4.32	1.8	4.2
	.....	.....	.....	.....	.....	.....	16-19	3.77	1.4	4.8
Wind deposit above this line; old normal profile below										
A.....	45-48	7.74	2.5	30-33	6.85	2.1	19-22	6.63	2.8	4.6
A.....	48-51	7.02	2.7	33-36	6.67	2.2	22-25	6.49	2.4	4.6
A.....	51-54	6.91	2.6	36-39	6.57	2.0	25-28	6.48	2.9	4.6
B.....	54-57	7.27	2.6	39-42	6.08	2.0	28-31	7.17	3.0	4.0
B.....	57-63	6.90	2.4	42-48	5.89	2.0	31-37	5.76	2.4	5.2
B.....	63-69	6.54	2.0	48-54	6.17	1.7	37-43	5.74	2.2	5.2
B.....	69-75	6.15	1.7	54-60	5.47	1.7	43-49	5.17	2.0	5.8
C.....	75-81	4.62	1.3	60-66	3.13	1.2	49-55	3.57	1.4	5.8

\* ME, moisture equivalent; LI, loss on ignition.

lenses of coarse material a few inches thick were found at varying depths.

The fine earth fraction was analyzed by the BOUYOUKOS (4) hydrometer method, and the total sands, "total colloids," and clay determined. Silt was determined by differences. The sands were recovered, and after drying were separated by sieves into the fractions shown in table 11. The fraction 0.25-0.05 mm. was determined by difference.

On the whole data from the mechanical analyses are not very helpful in furnishing an explanation of the problems suggested. No significant correlations have been established for differences in the sand fractions. The total colloids do vary in value with depth and among the various communities. Differences between the profiles from the *Trichostema-Andropogon* community and those of the closed communities are more probably the result than the cause of the difference in wind erosion, however, for comparable horizons at lower depths do not show significant differences. It is obviously impossible to find a value for the colloidal content of the horizons which were eroded away. That the differences which exist at present in the surface layers of the soils in various communities are of some significance in determining the character of the present vegetation seems reasonable. Comparison of the moisture equivalent and loss on ignition values in various communities brings out the relative differences in inorganic colloidal content primarily responsible for the greater water-holding capacity of some horizons and profiles. It can be assumed that these differences might also be responsible for a larger nutrient-holding capacity in the sands with higher colloidal content, with corresponding effect upon growth and density of the vegetation.

LOSS ON IGNITION.—For coarse sandy soils, loss on ignition is usually regarded as a fair index of the organic matter content. Such soils usually contain little carbonate, and the loss caused by colloidal dehydration is small.

Soil samples were oven-dried to constant weight in crucibles at 110° C. They were then heated to redness in an electric muffle until all organic matter had been destroyed, and after cooling, loss of

weight was determined. The data are presented in tables 11, 12, and 13.

With the exception of the surface 1-3 inches, which contain recent accumulations of organic matter, comparable depths and horizons in the various communities, especially among those with a normal soil profile, show remarkably close agreement in ignition loss. This is well brought out in columns 3, 4, 5, and 6 in table 12, the last three columns showing almost perfect agreement in this property. A comparison of columns 3 and 4 indicates a slightly greater organic content in the community carrying the heavier cover of *Andropogon*, although the differences are not so striking as are those for moisture equivalent. A comparison of these values with those in table 13 from the uncultivated and buried profiles in the *Polygonella-Quercus* community shows rather good agreement for comparable levels, except in the upper 3 inches. There is a slight suggestion of some loss of organic matter in the lower layers of the cultivated profiles, but the data are not sufficient to warrant a definite conclusion. Such a loss also seems to be indicated for the soils on which the *Hypericum* community is growing and might be expected, for there has been little addition of organic matter to the barrens soils in the past 100 years or more. It seems more reasonable to assume such a loss than to assume that the present differences date back to the time of cultivation.

Low values were expected in the *Trichostema-Andropogon* community since no A horizon is present. The high surface value is due to mechanical burial of wind-borne dust. Bearing in mind that approximately 12 inches of sand had been removed by wind erosion from the profiles for which data are given in column 1, table 12, and comparing the various depths with the comparable horizons in columns 3, 4, 5, and 6, agreement on the whole is good, the sharp break in all series occurring at the C horizon.

The low and fluctuating values of ignition loss in the wind-borne deposits were expected, since rapidity of sand deposition and amounts of plant parts buried would vary with both time and place. The data presented are all from profiles in dunes which carry little undergrowth at present. Higher values at the surface were mainly caused by the presence of organic dust. Areas with moss and lichen

mats would undoubtedly have higher surface values than those recorded.

Consideration of all the data for ignition loss seems to indicate that the ranges in value are most easily interpreted on the basis of the presence or absence of erosional and depositional phenomena following an original cultivation, mentioned many times previously, combined with recent surface depositions of amounts varying among the communities. In contrasting the profiles from barrens with those from other communities, it seems obvious that differences in organic matter content of the various surface soils resulting from erosion and deposition have been important in the establishment or failure of certain species of plants. Although no tests were made, the differences are apparently great enough to cause significant variations in ammonification and nitrification rates.

**MOISTURE EQUIVALENT.**—This value is often considered to be the most important of the “soil moisture constants,” and is regarded by some soil scientists as one of the most significant single-value determinations of soil properties. Not only is it a useful laboratory measure of water-holding capacity, but in conjunction with loss on ignition data it gives an indication of the inorganic colloidal content of coarse sandy soils. Moisture equivalents of pure sands are very low, often less than 2 per cent, and any marked increase over such values is often related to the colloidal content, both organic and inorganic. When two sands similar in texture in the coarse fractions and practically equal in organic content show marked variation in moisture equivalent values, it may be assumed that the differences indicate corresponding fluctuations in the inorganic colloid content. Such variations are significant in their bearing upon the relative adsorption and retention of mineral nutrients by the two soils.

Since moisture equivalent determinations can be made rapidly, a means is afforded of securing considerable applicable data in a relatively short time; hence this property was investigated extensively. Determinations were made in the usual manner, and loss on ignition values were secured on the same samples. Many of the data are presented in table 12. This value is also recorded in tables 11 and 13.

The results are in general agreement with those of the loss on

ignition determinations, although the differences among the various profiles are more pronounced. It is probable that the greater density of *Andropogon* and the greater number of forbs on those areas possessing moisture equivalent values of the order indicated in column 3, table 12, over those with the values shown in column 4, are related to the greater inorganic colloidal content indicated in the former. The slight differences in ignition loss would suggest that a difference in organic colloidal content is not responsible for the greater water-holding capacity in the first case.

The determinations for the barrens communities, correlated with the eroded or buried types of profile, show the low moisture equivalent values which would be expected, re-emphasizing the unfavorability of the present surface soils in such communities for plant cecesis.

ACIDITY.—Determination of soil acidity is useful for several reasons. Acidity values in the various horizons indicate approximately the degree to which the chemical profile is developed. So far as plant growth is concerned, one need not assume a toxic action of the hydrogen ion when in high concentrations to account exclusively for the poor growth often shown. The general correlation of low pH values with low available calcium, high soluble aluminum, decreased nitrification, and other factors is well known. Any or all of these factors may be responsible for the low productivity of highly acid soils, and the relatively limited flora often found on them.

The hydrogen ion concentration of the sand plains soils was determined electrometrically, with the quinhydrone electrode, on air-dried samples in the laboratory. Field determinations were also made, using the Morgan-LaMotte colorimetric method. Determinations by the two methods showed close agreement. No significant differences could be found among the various profiles. Some of the data are presented in tables 11 and 13. It should be noted that the soils are decidedly acid and that there is a general decrease of acidity with depth in truncated and normal profiles. The buried profile still seems to show normal stratification. The wind deposit shows the fluctuation with depth which one might expect on the basis of differences in organic content, rate of deposition, etc. The lowest pH values were found in the H horizons of the duff in the pine and



oak forests, suggesting a podzolizing effect of this duff upon the lower layers. It cannot be assumed that differences in soil acidity are causative factors in the local distribution of vegetation types upon the plains at present.

### Discussion

Ecologically, the sand plains study has been significant in illustrating the importance of historical factors in a causal analysis of the pattern and kinds of present-day plant communities. A complete and satisfactory explanation of the present sand plains vegetation can be arrived at only if the profound influences which these factors have exerted are kept in mind. Likewise the pattern of edaphic habitats can be appreciated only upon such a basis.

Although the present study indicates that existence of the barrens is of much shorter duration than the ages which have apparently been assumed by previous investigators, one may ponder the very slow rate of successional change in the extremely open *Trichostema-Andropogon* community. In spite of complete destruction of the A horizon, the sere should probably still be regarded as secondary. Persistence of an open pioneer subsere stage for 125 to 150 years is not usual in a deciduous forest climate. It emphasizes the profound importance of soil conservation, and the relatively permanent effect which soil profile truncation may produce.

It is possible and even probable that the *Andropogon-Cladonia* association as found at present is a type of plant community which had no extensive development prior to white occupation. It is my opinion, arrived at through a study of the fence row relicts, that the original grassland vegetation showed less development of lichens and much greater richness in forbs. The forbs were largely eliminated by cultivation and only a few have been able to spread back into the abandoned cultivated fields, while the lichens, with their better means of dispersal, have been able to assume a much more important role. The persistence of both the original grassland and the *Andropogon-Cladonia* association in the area probably was, and is, caused largely by recurrent fires. Burning during the resting season, aside from killing many of the woody invaders, is probably more helpful than harmful to the grass. *Andropogon* apparently develops

flowers and fruits to a much greater extent following such fires than when unburned, a fact which may be important in maintaining the stand in a youthful condition. No attempt has been made to explain this fact.

With the cessation of fire, the succession to the oak forest, as indicated in figure 5, will probably go on as rapidly as the conditions of seed dispersal permit. Man could do much to hasten this process.

From the economic point of view, a large part of the sand plains area, both in its past history and in its present state, furnishes a striking and early example of the wastefulness of a *laissez faire* land policy. Although the more intelligent and more experienced of the original settlers undoubtedly had some idea of the relative values of land types in agricultural practice, the method of land allotment and the general conditions in the pioneer communities made it inevitable that an attempt would be made, by people of lesser knowledge and experience, to cultivate these level terraces of coarse sand. The squatter was not unknown in colonial New England, in spite of the strict ideas and laws regarding land ownership, and in some cases allotment seemed only to legalize the possession of lands which had already been occupied. Granting that cultivation and subsequent abandonment were inevitable, the state of affairs which has existed since is not one of which the successive owners should feel proud.

A logical and complete scheme of land utilization should involve the growth of forests upon the areas under consideration. In those areas which still retain a normal soil profile, the meager data furnished by the black oak plantings, the abundant natural reproduction of pitch pine in local areas, and the relatively vigorous growth rates of both species when once established, suggest that one or both of these native species could be used successfully in silvicultural practice. Whether forests of these species would pay from the economic point of view is questionable. If ravages of the borer can be overcome, black locust would also be a desirable species for this area. Its growth rate on the plains is fairly rapid, and the possibilities of using the wood for fence posts at a relatively early age might make plantations profitable. More important, this species would improve nitrogen relations of the soil (12), and thereby have considerable

influence on future plantings. Red pine also would grow on the soils with a normal profile, although the site index for this species is low on Merrimac and Hartford soils in Connecticut (33). Any or all of these species could be used successfully in reclaiming much of this area.

The areas in which profile truncation has occurred present a more difficult problem. Observation, the planting experiments, and some of the factor measurements would indicate that an increase in the colloidal content of the soil is needed before ecesis of a large number of species and individuals could be assured. Discussion as to whether or not it is possible to bring about such an increase in a practical manner, and thus hasten the natural but slow process of plant succession and biotic reaction, is beyond the scope of this paper.

### Summary

1. The plant communities lying above the influence of the ground water table on coarse sandy terraces in the townships of Wallingford and North Haven, Connecticut, are mainly stages in three subseres which probably lead up to a xerophytic oak edaphic climax.

2. The subseres are developing on: ridges of wind-deposited sand, superimposed on a grassland type of soil profile; level areas with a truncated soil profile, lacking an A horizon; level areas with soil profile intact.

3. Differentiation of initial habitats resulted from, and followed, cultivation and wind erosion of the area in the eighteenth century.

4. Vegetation on the ridges consists of trees, primarily black oak and gray birch, forming an open canopy, with a scattering undergrowth of forbs, especially *Polygonella articulata*, grasses, shrubs, and occasional mats of mosses and lichens. Truncated soils have an extremely open cover of *Andropogon scoparius* and *Trichostema dichotomum*. Increase in abundance and cover is brought about chiefly through unintentional burial of seeds by man.

5. Normal soil profiles for the most part carry a complete cover of vegetation, usually a community of *Andropogon scoparius*, *Cladonia* spp., and *Polytrichum piliferum*, although occasionally the cover consists primarily of *Hypericum gentianoides*. Forests of black oak and pitch pine, and scrub and woodland of various species also

occur, and are mainly successional stages following the *Andropogon-Cladonia* association. Black oak invades many communities through burial of its acorns by squirrels.

6. Mechanical analysis, moisture equivalent, loss on ignition, and acidity values of the soils of various communities show differences related primarily to the presence or absence of erosion and deposition phenomena. Evaporation rates, maximum surface soil temperatures, and available soil moisture in summer, are highest in the *Trichostema-Andropogon* community and lowest in the forest communities.

The writer gratefully acknowledges the direction and encouragement of Professor GEORGE E. NICHOLS, Yale University, during the progress of this work. He also wishes to express his appreciation of the courtesies and aid extended by Professors ALEXANDER W. EVANS and HAROLD J. LUTZ, and Dean HENRY S. GRAVES, of Yale University; by Mr. M. FRANCIS MORGAN, Connecticut Agricultural Experiment Station; by Mr. CHARLES A. WEATHERBY, Gray Herbarium; and by Mrs. AGNES CHASE, Bureau of Plant Industry.

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# HISTOLOGICAL RESPONSES OF *IRESINE LINDENII* TO INDOLEACETIC ACID<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 484

BERTRAND F. HARRISON

(WITH NINETEEN FIGURES)

## Introduction

Numerous studies of the gross responses of plants to applications of indoleacetic acid have been made in the past few years, but few studies have been concerned with the specific tissues involved. The responses of some of the tissues of *Coleus* stems treated with indoleacetic acid in lanolin were mentioned and pictured in the studies made by LAIBACH and FISCHNICH (7). FISCHNICH (4) found in treated *Coleus* stems that the cells of the parenchyma enlarge, the cells in the vicinity of the vascular bundles proliferate, the cambium becomes active, and new vascular bundles appear between the old ones. He found that the roots arise principally in the borders of the vascular bundles. CZAJA (3) investigated the effects of indoleacetic acid-lanolin paste on *Helianthus* hypocotyls, applying it as a ring encircling the hypocotyl, unilaterally, and apically on the cut surface of decapitated hypocotyls. Treated areas were sectioned and some of the tissue responses were described. Similar studies were made of *Pisum sativum*. SNOW (9) reported a definite stimulation of cambial activity in *Helianthus* hypocotyls treated with indoleacetic acid in gelatin. A detailed study of the histological responses of Red Kidney beans treated with this acid was made by KRAUS, BROWN, and HAMNER (6). Later this study was extended and the responses of beans to other growth promoting substances were also investigated (5). BORTHWICK, HAMNER, and PARKER (1) have studied the histological and microchemical reactions of tomato plants to indoleacetic acid.

The stems of bean and tomato each represent distinct anatomical patterns. Because *Iresine lindenii* Van Houtte has a pattern differ-

<sup>1</sup> Additional cost of publication sustained by the writer.



ing markedly from either of these two, it was chosen for the investigation here reported. This species evidently includes the garden forms *I. emersonii*, *I. collensia*, and *I. formosa*. It is a small erect herb belonging to the Amaranthaceae. Native to Ecuador, it is widely cultivated in this country as a bedding plant, owing to the bright red color of its leaves and stems.

This investigation was begun in July, 1936, and continued for a year. The plants were grown in the greenhouse under favorable conditions, but no attempt was made to provide constant conditions of light, temperature, or atmospheric humidity. Cuttings were started from four months to about a year before the time of treatment. No differences were noticed in the types of responses of the stems at different times of year, but there was a marked difference in the rate at which the reactions occurred. Reaction was rapid in warm moist weather and abundant sunlight.

The earlier experiments consisted in the treatment of cuttings with aqueous solutions of indoleacetic acid, then planting them in clean quartz sand both with and without bottom heat. In making up the solutions, a few milliliters of alcohol were used to dissolve the indoleacetic acid crystals before water was added. The control plants were treated with water containing an amount of alcohol equal to that in the indoleacetic acid solution.

The treatment of cuttings with aqueous solutions of indoleacetic acid stimulated the proliferation of some of the tissues and the production of numerous adventitious roots. It was found, however, that if the indoleacetic acid was applied to stems in a mixture of lanolin the tissue responses were localized in a much shorter portion of the stem. Since this method lends itself more readily to a histological analysis of the tissue responses, it was adopted for all subsequent experiments.

A mixture of 3% of Merck's crystalline indoleacetic acid in anhydrous lanolin was used in all cases. Two types of applications were made on each of two internodes of different degrees of maturity. The first obviously elongated internode down from the tip which had reached a length of 6-10 mm. is recorded as the first internode, and the second internode below the first is recorded as the third internode. One method of treatment consisted in encircling the

upper-middle portion of any given internode with a narrow band of the lanolin mixture, or of pure lanolin in the case of control plants (fig. 1A). Care was taken not to injure the stem otherwise. Not more than one internode was treated on any given stem. The other method of treatment consisted in decapitating the stem at the upper part of the first or third elongated internode, blotting the surface with absorbent paper, and applying the mixture or pure lanolin to the cut surface (fig. 1B, C).

Specimens were taken at various intervals, fixed in CROOKS modification (2) of Navashin's fluid, and imbedded in paraffin for sectioning. Sections were cut  $10\mu$  thick. The interpretations are based upon the examination of several thousand sections from plants of different ages and material in several stages of development.

### Gross responses to treatment

#### AQUEOUS SOLUTIONS

At the beginning of this work a number of stem cuttings were treated with aqueous solutions of indoleacetic acid ranging in strength from 0.05 to 0.5 mg. per milliliter for fourteen hours. Even the weakest of these concentrations proved highly toxic. The average number of roots produced by the individual cuttings was in inverse proportion to the strength of the solution with which they were treated, the untreated controls showing the greatest number of roots. Subsequent trials showed that cuttings treated with a solution containing 0.1 mg. indoleacetic acid per milliliter of water for three and a half hours produced an average of about five times as many roots as did untreated controls, by the end of two weeks. Untreated stems produced roots only at the lowest node and within 2 or 3 mm. of the cut end of the internode. Treated plants frequently produced an abundance of roots the entire length of the lower internode.

#### INDOLEACETIC ACID-LANOLIN MIXTURE

First internodes which were treated with a ring of lanolin mixture began to swell soon after treatment. By the end of thirty hours the portion of the stem under the ring and adjacent to it had swelled noticeably and become somewhat lighter in color. During this time

the nearby leaves bent and twisted, although later they usually straightened out again. The stem may also bend and twist and may remain crooked. The treated area continued to increase in size slowly and rather uniformly for about ten days, after which there was little further enlargement. Meanwhile the stem, a short distance above and below the lanolin ring, formed a spindle-shaped tumor. The response was usually confined to a few millimeters on each side of the band, but sometimes the whole internode became involved. Roots penetrated the surface of the stem about ten days after treatment. They may continue to grow until they are about 4 or 5 mm. beyond the surface of the stem, when they usually dry up. If the atmosphere is humid they continue to elongate. Since there are numerous roots formed, the cortex may be partially lifted off or torn; the surface of the stem thus becomes rough and broken, or a number of longitudinal cracks may be formed. Previous to the appearance of roots the surface of the swelling is usually ridged, the ridges resulting from the enlargement of the collenchyma of the cortex.

Control plants in which the first internode was ringed with pure lanolin showed a very slight enlargement under the ring. No roots were formed nor did the swelling extend perceptibly beyond the limits of the lanolin.

Third internodes which were ringed with indoleacetic acid mixture showed very local swelling confined to a few millimeters in breadth. There is considerable difference in the state of maturity of these so-called third internodes, so there were some differences in the reactions of different stems. The less mature stems showed more obvious responses than more mature ones. The enlarged areas were not noticeably ridged nor broken except where roots penetrated the surface. Root primordia appeared in ten to twelve days. There were fewer roots formed than in the first internode; those which were formed behaved much as did those of the first internode. Not all of the roots formed penetrated to the outside; some grew downward just inward from the collenchyma of the cortex.

Control plants in which the third internode was ringed with pure lanolin showed almost no responses which could be detected with the unaided eye.

The first noticeable response of decapitated first internodes to applications of lanolin mixture to the cut surface was a slight swelling of the stem immediately below the treated surface about thirty hours after treatment. The stem continued to increase in diameter,

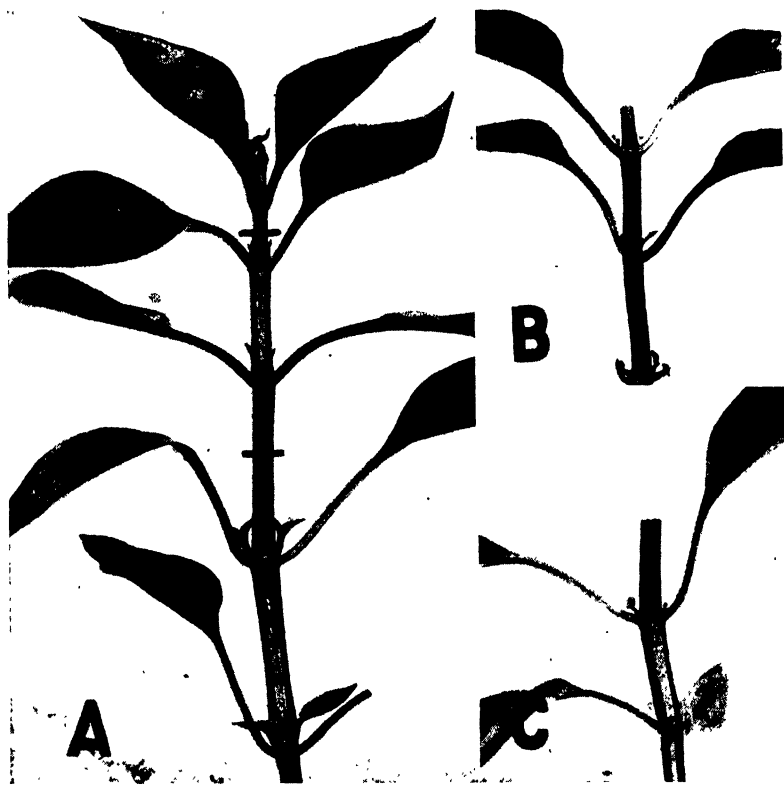


FIG. 1.—Stems of *Iresine* at beginning of experiments. A: lines indicate portion where indoleacetic acid in lanolin would be applied encircling first or third internode. B: decapitated first, and C: decapitated third internode with layer of lanolin applied to cut surface.

the greatest swelling taking place about 2 mm. below the cut surface. There was no development of tissue above the original level of the cut surface and less than the first centimeter of the stem was affected by the treatment. The surface of the swelling may be ridged, but usually it remained rather smooth.

The root primordia penetrated the epidermis just below the cut

surface about nine days after treatment. An almost complete ring of roots was usually formed, and in this area the cortex was lifted off the stem (fig. 3A). The dried fragments may remain attached to the edge of the intact cortex. As in laterally treated stems, the roots grew out from the surface 4-5 mm. and then dried up and



FIG. 2.--Stems 360 hours after ringing with lanolin mixture. A: first internode; B: third internode.

ceased to elongate unless kept in a moist atmosphere. A few stems were observed for about a month after treatment; after about fourteen days little change was noticed in their external appearance.

First internodes which were decapitated and pure lanolin applied to the cut surface showed a very slight swelling immediately adjacent to the cut surface. No subsequent changes were observed.

Decapitated third internodes treated with lanolin mixture showed essentially the same responses as did treated first internodes, except that the stems were much less reactive. The swellings were smaller in proportion to the size of the stem, and the number of roots pro-

duced was usually not so great. Within about thirty to forty hours the stem began to swell a few millimeters below the cut surface. This continued until the enlarged portion was about double the diameter of the original stem. There was little further change until the roots emerged after about twelve days (fig. 3*B*). The cortex was



FIG. 3.—Decapitated stems 312 hours after lanolin mixture applied to cut surface. *A*: first internode; *B*: third internode.

torn loose where the roots emerged and the roots reacted as in the other cases.

The decapitated third internodes which were treated with pure lanolin as controls showed no marked responses except slight enlargement immediately adjacent to the cut surface.

### Histological details

#### NORMAL ANATOMY

Transections of the first internode of *Iresine* stems are somewhat oval in outline. Two prominent opposite ridges which extend the

length of the internode alternate with two lateral ridges. A group of three primary vascular bundles is located in each of these four ridges, and through part of the internode four additional bundles may alternate with these four groups, making a total of twelve to sixteen bundles (fig. 4A). The bundles may anastomose near the node but they do not become united laterally to form a continuous cylinder of vascular tissue. WILSON (10) has described the anatomy of *Iresine paniculata*, and his description applies to this species as well. Two or three layers of collenchyma occur subepidermally in each of four ridges. Between the ridges the cells are thin-walled and contain numerous chloroplasts. There are seven to ten layers of cortical cells but no well defined endodermis.

In young stems the pericycle appears as a nearly continuous band one cell in thickness. Divisions in a centripetal direction produce at intervals cells which mature to form small bundles of functional xylem. Between these bundles the cells formed by the pericycle and its derivatives become uniformly thickened, and at maturity appear nearly square in transection and rectangular in longitudinal view. WILSON calls this conjunctive tissue and attributes it not to a pericycle directly but to an extrafascicular cambium formed by the pericycle. After a small bundle of xylem cells has been formed, the derivatives of the pericycle external to the bundle function as a fascicular cambium and divide centrifugally to form a number of cells which mature as phloem. On each side of the bundle additional layers of conjunctive tissue are laid down. Later the bundle may become completely imbedded in this tissue. The number of layers of thickened cells varies with different plants for corresponding internodes and also with the level in any given internode. The tissues in the upper portion of an internode are the most nearly mature since the base of each internode remains relatively meristematic. In the older internodes the bundles inward from the conjunctive tissue may be separated from it by several layers of cells of primary origin. This condition is seen especially at the middle of the internode.

Adventitious roots develop from untreated cuttings within six to ten days after they are planted in moist sand. The roots arise from very near the cut surface at the base and from the internode

near the lower node or nodes if more than one node is buried in the sand. Scarcely any roots arise from the internodes of untreated stems except from that portion which is very near the cut surface or a node. The tissue from which the adventitious roots arise is the pericycle and its undifferentiated derivatives. The first divisions in the formation of the roots usually occur in the outer layer of cells; that is, in the pericycle, and soon afterward the next layers inward also become active. From the cells which arise from the first layer there is organized a calyptragen from which the epidermis and the root cap are developed. The histogens for both the periblem and the plerome appear to originate in cells derived from the second layer; that is, the one immediately underlying the pericycle. The layers of cells inward from these outer layers divide actively and form a number of cells which make up that part of the root nearest the center of the stem. Root primordia often arise external to the small secondary bundles imbedded in the conjunctive tissue, in which case the phloem of these bundles becomes active and is involved in the formation of the lateral roots.

The principal responses of the various tissues to treatment are illustrated by means of photomicrographs. These show some of the more obvious changes which were observed.

#### RESPONSES OF FIRST INTERNODES ENCIRCLED WITH LANOLIN MIXTURE

Following lateral applications of lanolin mixture to the first internodes, the cells of the epidermis become very reactive. Soon after treatment they enlarge, principally in a radial direction (fig. 4*B*), and after considerable expansion divide, mainly tangentially. In local areas the epidermal cells divide repeatedly, forming small protuberances of cells which project beyond the neighboring ones. By the end of 216 hours practically all the epidermal cells in the treated area had divided tangentially at least once (fig. 7*A*). Subsequently a new epidermis is organized from the outer layer of daughter cells.

The cortical collenchyma is the first tissue to show noticeable responses to lateral treatments. Within thirty hours the cells had begun to enlarge. Enlargement continues to such an extent that



the cells lose their characteristic appearance as collenchyma, the walls become distended, and the thickenings disappear. There are scarcely any intercellular spaces (figs. 4*B*, 5*A*). The cytoplasm of the cells becomes dense and many of the cells divide, mainly tangentially. Some of the cells respond much more readily than others, but divisions of the cells of the collenchyma are never numerous.

The cells of the cortex, exclusive of the collenchyma, show little response except a slight general enlargement. By the end of 168 hours there may be a slight increase in the number of divisions over that of untreated stems, especially in the inner cortical cells (fig. 7*A*). There is no well defined endodermis but such as is present shows only slight response. External to young root primordia the cells may divide a number of times, but ordinarily they are only slightly more active than the other cells of the cortex.

The first cells of the pericycle to respond are those which occur external to the vascular bundles (fig. 4*A*). Soon, however, the pericycle cells which lie interfascicularly also proliferate, and a continuous band of meristematic cells is established (fig. 6*B*). The cells of the meristematic layer are involved in the initiation of root primordia but to a lesser extent than the cells of either the phloem or rays.

Of all the first internode tissues the phloem is the most responsive to treatment. Beginning soon after treatment the cells enlarge in all directions, especially radially. At the outer periphery of the bundles a large cap of highly meristematic cells is formed from the phloem, pericycle, and in some cases from the cells of primary origin which lie between the phloem and the pericycle (fig. 5*A*). After the extrafascicular cambium becomes active its newly formed derivatives contribute to this mass of cells, which lie just inside it. The tissues exterior to this cap of cells are pushed outward and laterally. The sieve tube-companion cell groups become widely separated from one another, and some time after treatment they appear scattered throughout the mass of proliferating cells. The cells of this active tissue contribute to the formation of the adventitious roots.

While the fascicular cambium remains active following treatment with indoleacetic acid, it does not appear to be markedly stimulated. The cambial zone remains rather narrow, and as a result

most of the sieve tubes and companion cells do not become separated from the xylem by a very wide zone (fig. 7*A*, *B*). No divisions were observed in the cells of the xylem parenchyma although they may enlarge slightly.

The cells of the pith, except those of the rays, are affected little or not at all by lateral treatments. In a few stems some of the pith cells appeared slightly enlarged, but no other changes were observed in the pith at any level of the treated stem.

The ray tissue lying between the bundles and especially that immediately adjacent to the phloem responds more actively to treatment than any other tissue except the phloem. The ray cells are among the first to become active, and this activity spreads laterally until there is a band of meristematic cells between each pair of bundles (fig. 5*A*). This meristematic band becomes broader by subsequent divisions of the cells composing it, and by divisions of the pericycle cells, until a broad zone of meristematic cells is formed extending from the level of the fascicular cambium outward to the endodermis. The endodermis is finally pushed outward, well beyond its former position (fig. 7*A*).

It is from this zone of meristematic cells both over and between the bundles that the primordia of the roots arise. By far the greater number of roots originate in the ray cells immediately adjacent to the phloem, with the phloem, rays, and pericycle each contributing to their formation (fig. 6*A*). Occasionally roots develop from the ray and pericycle about equidistant between two bundles (fig. 6*B*). In the formation of the roots from first internodes it seems that the histogen of the calyptra arises from the pericycle and that the periblem and plerome arise from derivatives of the rays.

As the roots increase in length they push their way outward through the cortex. No evidence of enzymatic digestion of the cortical cells was observed; instead the cells are crushed and pushed together ahead of the lengthening root. Not all of the roots reach the surface; some grow downward or laterally through the cortex just within the collenchyma.

Some of the derived cells which lie inward from the root tip differentiate as xylem elements and phloem elements, so that the

vascular system of the young root becomes directly connected with similar elements of the vascular bundles of the stem (fig. 7*A*, *B*).

First internodes which were encircled with pure lanolin as controls resembled the treated stems in the activity of the epidermis. Epidermal cells of control plants enlarged and divided periclinally in the region where lanolin was applied, and 384 hours after treatment the majority of the epidermal cells had divided at least once. The epidermis was but slightly less active than in stems treated with the lanolin mixture. In figure 5 a stem 144 hours after treatment with the mixture is compared with a stem 384 hours after treatment with pure lanolin. The cells of the collenchyma in stems treated with pure lanolin also enlarged slightly although no divisions were observed. Through a very short vertical distance the other cells of the cortex may also become slightly enlarged. In none of the other tissues were any differences from untreated stems observed.

#### RESPONSES OF DECAPITATED FIRST INTERNODES TO APICAL APPLICATIONS OF LANOLIN MIXTURE

The epidermal cells of apically treated first internodes do not respond as in laterally treated stems. Those changes which do occur are found largely within 1 mm. of the cut surface, where the majority of the epidermal cells enlarge to about one and one-half times their original size but show no further obvious responses. Those immediately below the cut surface, and some in vertical strips, may enlarge to more than double their original size and divide as in laterally treated stems. Since this response is local rather than general, it may be because of lanolin coming in contact with the side of the stem.

Soon after treatment the cells of the collenchyma begin to enlarge, principally in a radial direction; subsequently a few of them may divide. At first only the cells immediately adjacent to the cut surface enlarge, but later cells farther down become involved although the region of activity never extends more than a few millimeters down the stem. As with the epidermis, there may be considerable increase in the size of the cells and active divisions in localized vertical strips of the collenchyma.

The inner or parenchymatous cells of the cortex show little change

for the first 72 hours following treatment, but later they enlarge and some of them divide. Not all of the cells are equally affected; in local areas the cells may divide repeatedly in all directions while in other parts at the same level they may divide but rarely. All intermediate conditions exist. The activity of the cortical cells is confined largely to the first 2 mm. below the cut surface. In the root zone much of the cortex is torn from the stem by the developing roots. No special activity in the endodermis was observed.

The pericycle is one of the more reactive tissues of the stem. Soon after treatment the cells enlarge slightly, the cytoplasm becomes dense, the nuclei become more prominent, and directly thereafter some of the cells divide (fig. 8*A*). As in laterally treated stems, the pericycle becomes active first over the bundles and later between the bundles, so that a nearly continuous band of proliferating cells is formed.

The cells of the phloem are extremely reactive. They may enlarge to more than double their former size before they divide. Following division the daughter cells enlarge and divide repeatedly. At the same time the cells of the rays begin to proliferate (fig. 8*A*, *B*), repeated divisions occurring centripetally. This often results in a long row of cells all derived from a single parent cell (fig. 9*A*). The proliferating cells of the phloem, pericycle, and rays contribute to the formation of a broad zone of meristematic tissue which extends as a continuous band outward beyond the original limits of the vascular bundles (fig. 9*A*, *B*).

The root primordia are organized from this zone of meristematic cells. As in laterally treated stems, the primordia arise mainly from the ray tissue and the pericycle, although some of the phloem derivatives may also be involved. The roots are frequently so close together that almost the entire band of cells is involved in root production, and fasciation is not uncommon (figs. 9, 10*B*). As the roots develop, an increasing number of cells within this band become active. There were numerous divisions in the outer cells of the pith. The cells of the pith frequently divide actively throughout the first millimeter below the treated surface, but few divisions were observed in the pith below this level except at its periphery. No cases

were observed in which the pith had proliferated sufficiently to project above the level of the cut surface.

The proliferation of the phloem cells and of the cambium derivatives forces the sieve tube-companion cell groups outward and separates them into smaller and smaller groups. The cells of the xylem parenchyma proliferate and spread the elements of the xylem apart. At the same time many of the ray cells differentiate as tracheids more or less at random (fig. 10C). These activities result in a nearly continuous band throughout which vascular elements are distributed, whereas the bundles of laterally treated stems remain sharply separated (fig. 10A, B).

The responses of decapitated first internodes to apical applications of pure lanolin consisted principally in a slight enlargement of cells of the cortex and pith near the cut surface. There was also some enlargement and radial division of some of the cells of the epidermis and collenchyma. In stems taken 384 hours after treatment these responses were confined to a vertical distance of less than 500  $\mu$  from the cut surface. All other tissues appeared as in untreated stems.

#### RESPONSES OF THIRD INTERNODES ENCIRCLED WITH LANOLIN MIXTURE

At the time of treatment of the third internodes, the pericycle and its derivatives appear as a continuous ring four to eight layers of cells thick, surrounding the stele. There is a marked difference in the state of maturity of these cells for different plants and at different levels in any given internode. In some third internodes none of these cells has any noticeable thickenings of the walls, while in others there are three or four layers of conjunctive cells with heavily thickened walls, and outward from these are three or four more layers in which the walls are not thickened. The responses of this latter type to treatment differ markedly from those of first internodes, but third internodes of the first type resemble more closely first internodes in their responses.

The tissues of the third internode, especially the more mature internodes, are much less responsive to treatment than those of first internodes. The epidermal cells of treated third internodes enlarge only slightly beyond their original size. Subsequently they

divide in regular tangential divisions with little further increase in size (figs. 12*B*, 14). The cells of the collenchyma respond much as in laterally treated first internodes but to a lesser extent. The cells enlarge about one and one-half times, principally in a radial direction. A small proportion of them divide.

The cortex, exclusive of the endodermis, shows but slight enlargement of the cells. The endodermis is reactive to the extent that there are occasional divisions, especially in the vicinity of developing roots.

The pericycle and its derivatives, together with the phloem of the secondary bundles, are the most responsive tissues. In stems in which the walls of the conjunctive cells have not become thickened, the entire band of conjunctive tissue, or nearly all of it, becomes meristematic and the tissues interior to it may also become involved (fig. 13*A*, *B*). The tissues inward from the conjunctive tissue which become active are the phloem, ray cells, and a cap of cells of primary origin which occur between the primary bundle and the conjunctive tissue. Cells of the pith are not noticeably affected.

In stems which have a number of layers of thickened conjunctive cells the responses to lateral treatments are confined to the non-thickened conjunctive cells and those external to them. Soon after treatment, the phloem of the secondary bundles and the nearby pericycle and its unthickened derivatives become active. Only rarely do any of the conjunctive cells with thickened walls enlarge or divide. Usually the first cells to respond are those which lie directly external to the primary bundles (fig. 12*A*, *B*). Later the remainder of the pericycle and its derivatives may proliferate, but it is in the vicinity of the primary bundles that most of the roots arise. Apparently the calyptragen develops from the pericycle and the periblem arises from the younger pericyclic derivatives which are still meristematic. The plerome seems to develop either from the young pericyclic derivatives or from the phloem of the secondary bundles (fig. 14).

As the lateral roots increase in length, some of the cells derived from the root tip differentiate as vascular elements. In stems in which there is little or no thickened conjunctive tissue, the cells lying inward from the root (including those of the conjunctive tissue)

may also differentiate as vascular elements and become part of the conductive system of the root. In such cases the xylem elements and the phloem elements of the young root subsequently extend through the band of conjunctive tissue and become connected with similar elements in the primary vascular bundles of the stem, although they may also become connected with the secondary bundles occurring in the conjunctive tissue (fig. 13*B*). In contrast to the condition just described, the vascular systems of roots which develop outside of a number of rows of thickened conjunctive cells do not become connected with the elements of the primary vascular bundles. Cells derived from the root tip differentiate as xylem and phloem elements adjacent to similar elements of the secondary vascular bundles, which lie in the band of conjunctive tissue (fig. 15).

Third internodes which were encircled with pure lanolin as controls differed only slightly from untreated stems. Most of the epidermal cells under the ring of lanolin became slightly enlarged and divided tangentially. A few of the epidermal cells 384 hours after treatment had divided several times. None of the other tissues in the control plants showed any noticeable differences from untreated stems.

#### RESPONSES OF DECAPITATED THIRD INTERNODES TO APICAL APPLICATIONS OF LANOLIN MIXTURE

Third internodes which were treated apically proved to be very reactive. They are not so reactive as first internodes but they show much greater activity than third internodes treated laterally. All tissues made up of living cells responded to some extent to apical treatments with the lanolin mixture.

Near the cut surface of the apex the cells of both the epidermis and the collenchyma become enlarged, although few of the cells divide. At distances of more than 1 mm. from the apex the cells of both tissues show no obvious differences from those of untreated stems. The cells of the cortex in small local areas show numerous divisions, but there is no general proliferation and such activity as does occur is confined to a very short vertical distance from the treated surface. As in the other treatments, the cells of the endoder-

mis may show a limited number of divisions but they do not become markedly meristematic.

The reactions of the other tissues are more or less a combination of the reactions manifested by laterally treated third internodes and apically treated first internodes. The pericycle and its derivatives begin to proliferate soon after treatment (fig. 16*A*). In most of the stems examined there was a rather general proliferation of this tissue throughout its whole circumference. Root primordia arise from the pericycle, the pericyclic derivatives, and the phloem of the secondary bundles as in laterally treated third internodes, but root primordia also arise from the pericycle and its derivatives where there is no phloem, especially in stems which have very little thickened conjunctive tissue (fig. 17*A*). Numerous root primordia were produced. More of the cells of the conjunctive tissue became active than in laterally treated third internodes.

All of the conjunctive cells with thin walls became active and proliferated extensively, and many of the conjunctive cells with thickened walls also divided extensively. The cells with thickened walls which did not divide were left as small isolated groups surrounded by proliferating tissue. Many of the conjunctive cells with thickened walls became differentiated as xylem elements with irregular wall thickenings. Some of the proliferating conjunctive cells differentiated as vascular elements which served to connect the vascular system of the young lateral roots with similar elements in the primary bundles. The vascular systems of a large majority of the lateral roots formed became connected with those of the primary bundles as described for laterally treated third internodes, but here also the vascular system of the young root may become connected with similar elements in the secondary bundles, at least in the case of the xylem. In the older stem sections examined there was considerable new xylem differentiated in the primary bundles from cells derived from the fascicular cambium. The xylem of many of the young roots became connected with this new xylem in the primary bundles. Some connections between the xylem of the secondary bundles and that of the primary bundles were established.

In contrast to the conditions found in laterally treated stems, those treated apically showed great activity in the tissues which lie



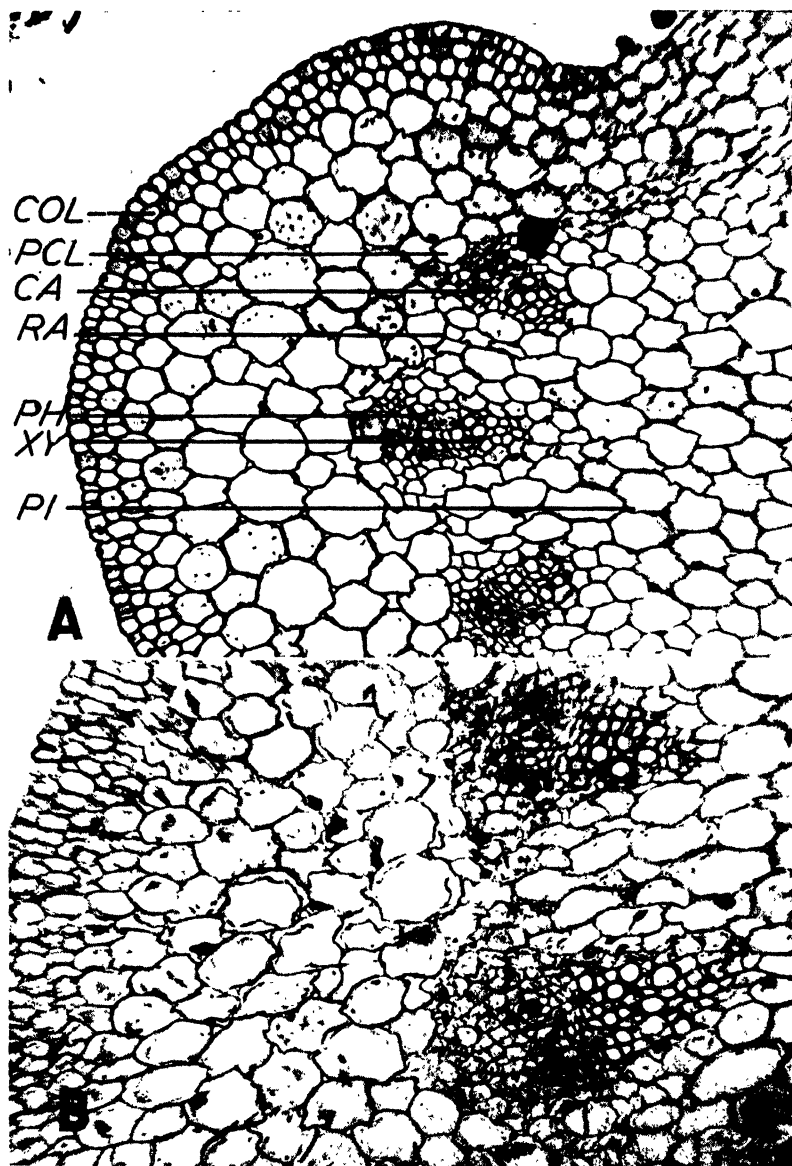


FIG. 4.—*A*: transection of first internode showing nature of tissues at beginning of experiments (*col*, collenchyma; *pcl*, pericycle; *ph*, phloem; *ca*, cambium; *xy*, xylem; *ra*, ray; *pi*, pith). *B*: first internode 110 hours after lateral treatment. Epidermis, collenchyma, and cortical parenchyma cells enlarged. Pericycle over bundles, phloem, cambium derivatives, and ray cells, especially those adjacent to bundles enlarged and dividing. Some xylem parenchyma cells enlarged slightly.



FIG. 5.—*A*: first internode 144 hours after lateral treatment. Phloem, pericycle, and ray tissue show increased activity. Root primordia have begun to be organized from ray tissue adjacent to each bundle. *B*: transection of control stem 384 hours after being ringed with pure lanolin. Epidermal cells have enlarged and divided tangentially; cells of cortex, especially those of collenchyma, have enlarged radially; other tissues as in normal stem of this age.

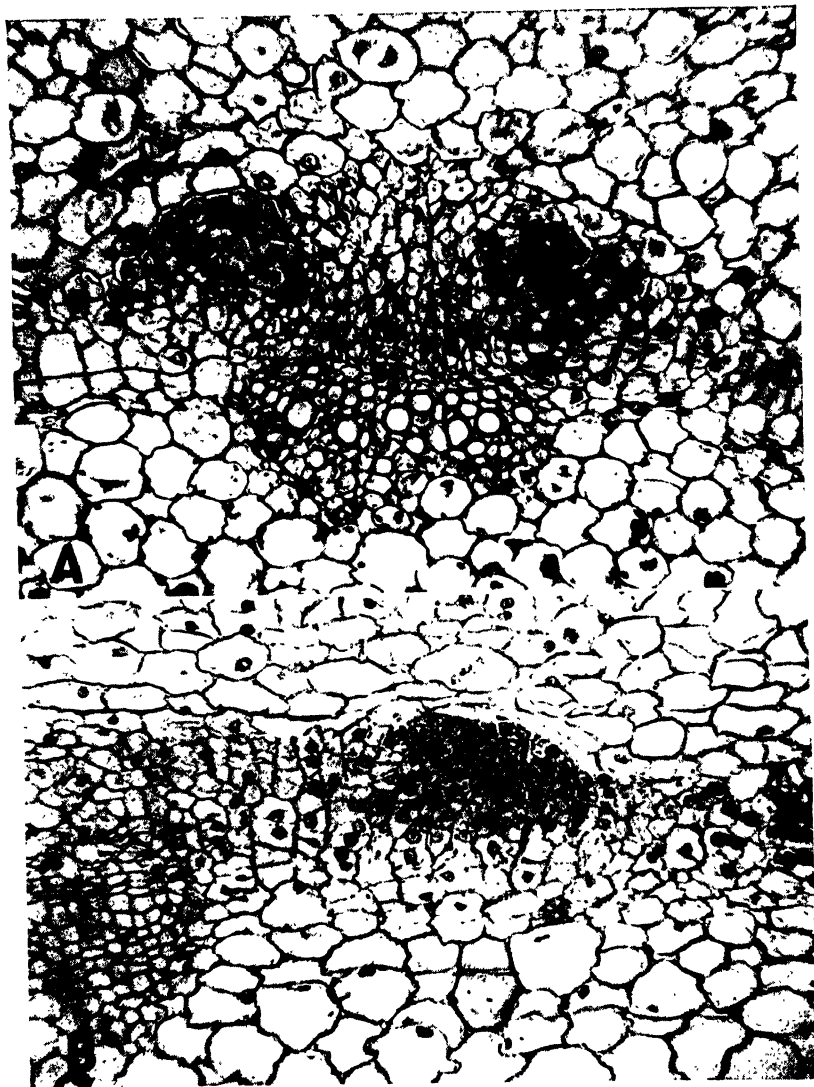


FIG. 6.—*A*: laterally treated first internode 144 hours after treatment, showing two root primordia developed from ray tissue and phloem of adjacent bundle. Pericycle and phloem of bundle active. *B*: origin of root from tissues between bundles; pericycle and ray tissue involved; 168 hours after treatment.

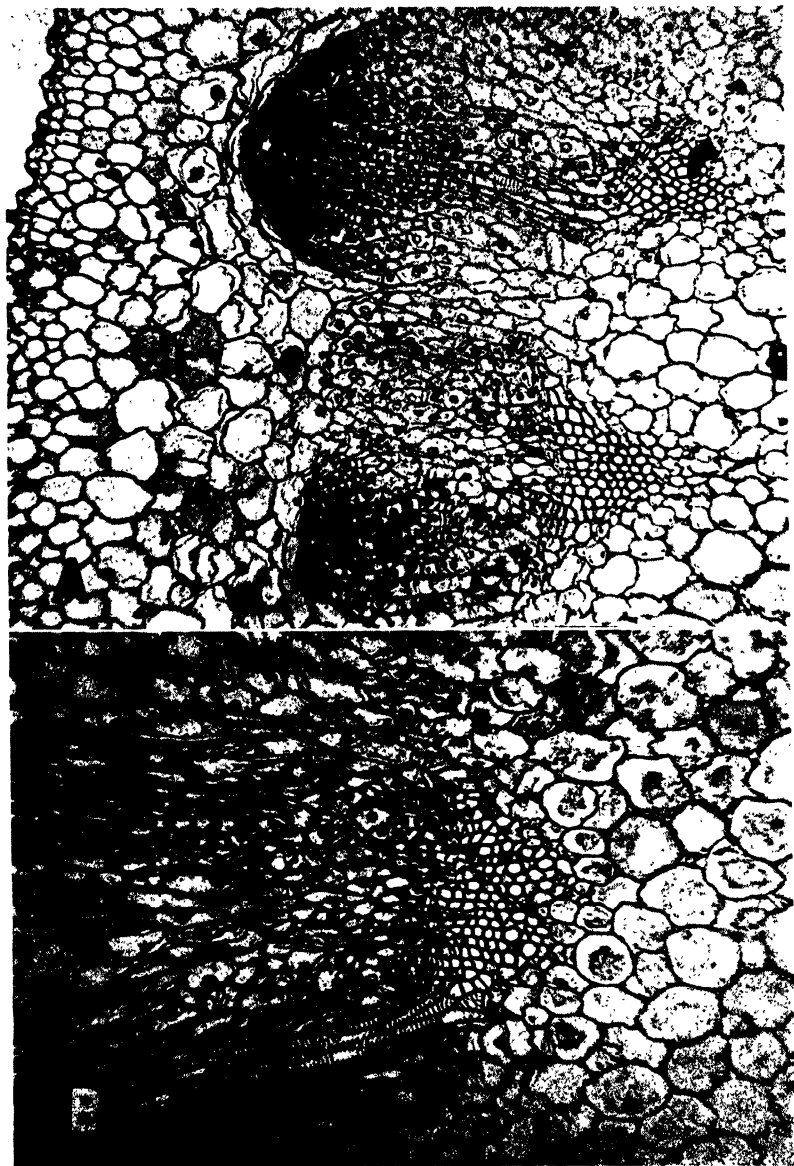


FIG. 7.—*A*: development of roots in stem 216 hours after treatment. Vascular tissue of root has differentiated back to that of bundle. Epidermal cells have divided tangentially. Phloem and ray cells have proliferated extensively. *B*: details of reactions 312 hours after treatment. Phloem and ray cells show great activity. Connection of vascular elements of root with that of stem is shown. Xylem parenchyma active; pith not meristematic.

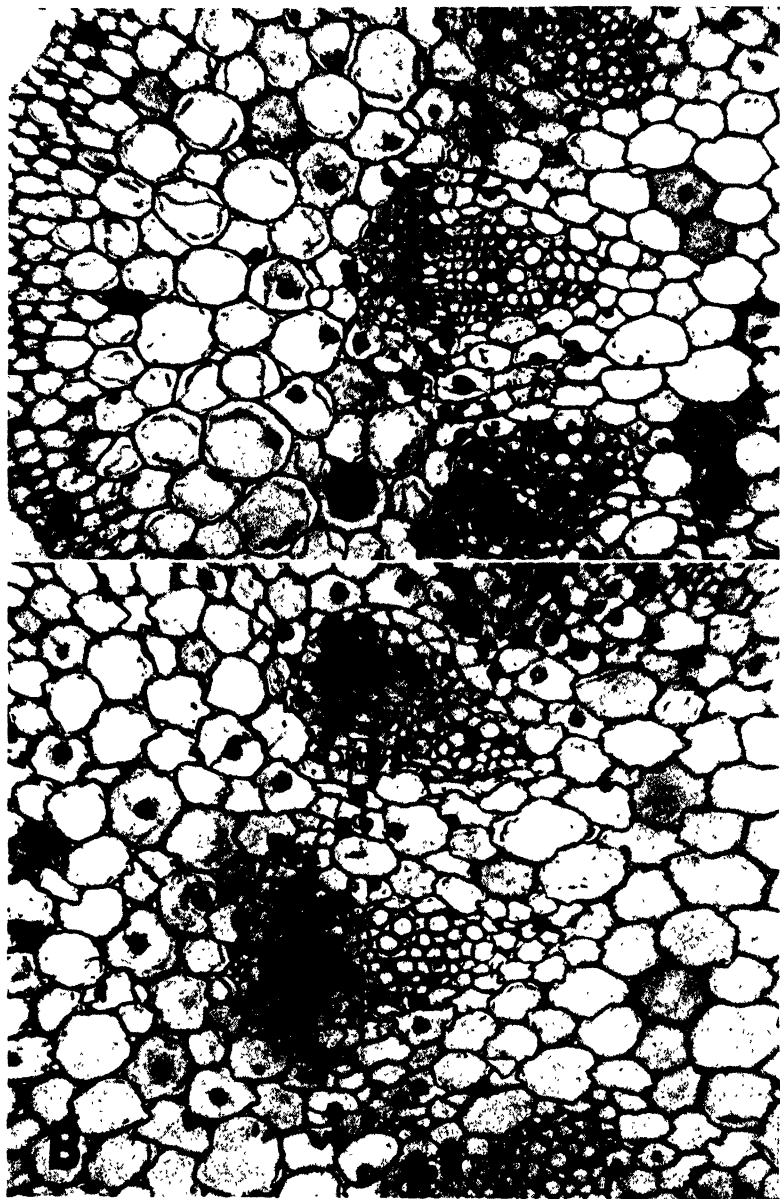


FIG. 8.—*A*: first internode 80 hours after indoleacetic acid was applied to apical cut surface. Pericycle, phloem, cambium derivatives, and ray cells show beginning of some activity. Epidermal and collenchyma cells show slight radial enlargement. *B*: 93 hours after treatment. Pericycle, phloem, and ray cells have begun to divide.

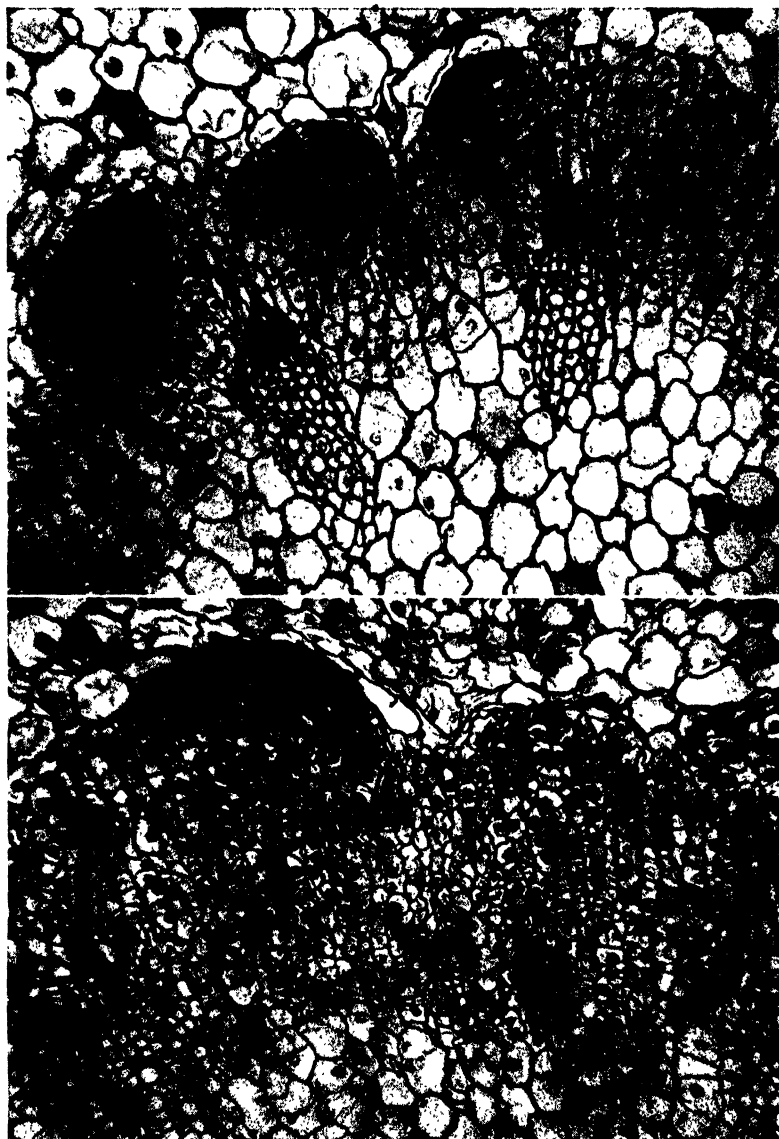


FIG. 9.—*A*: internode 144 hours after treatment. Root primordia organized from pericycle, phloem, and ray tissue; ray tissue especially reactive; xylem parenchyma slightly active. *B*: condition similar to *A* 168 hours after treatment. Ray tissue proliferated to form a nearly continuous band; activity extends farther into pith; phloem largely meristematic.

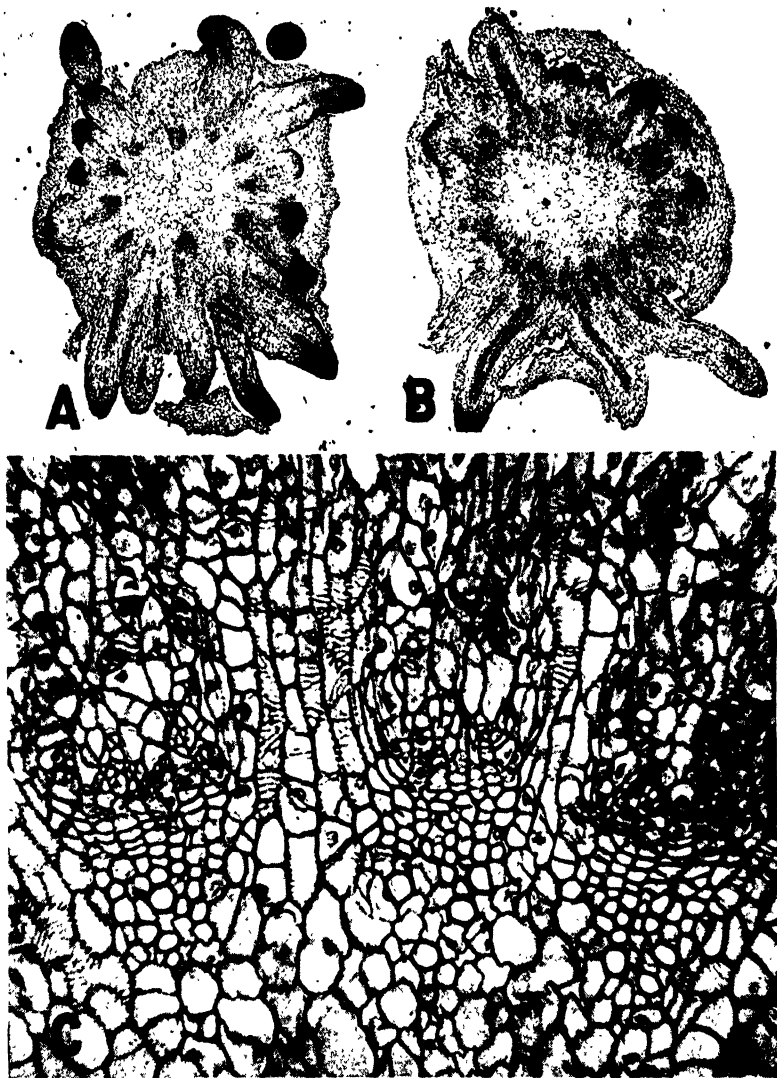


FIG. 10.—*A*: laterally treated first internode 312 hours after treatment. Roots lifting cortex free of stem in places; bundles still rather distinct; pith scarcely affected. *B*: apically treated first internode 312 hours after treatment. Pith rays more reactive than in *A*, giving rise to a broader and more nearly continuous band of meristematic tissue; bundles much less distinct; cortex less affected. *C*: details of region of bundles 312 hours after apical treatment. Numerous tracheids are differentiated; not all of them lead to roots. Phloem very active, ray tissue very active but activity does not extend far into pith (*cf.* fig. 6*B*).

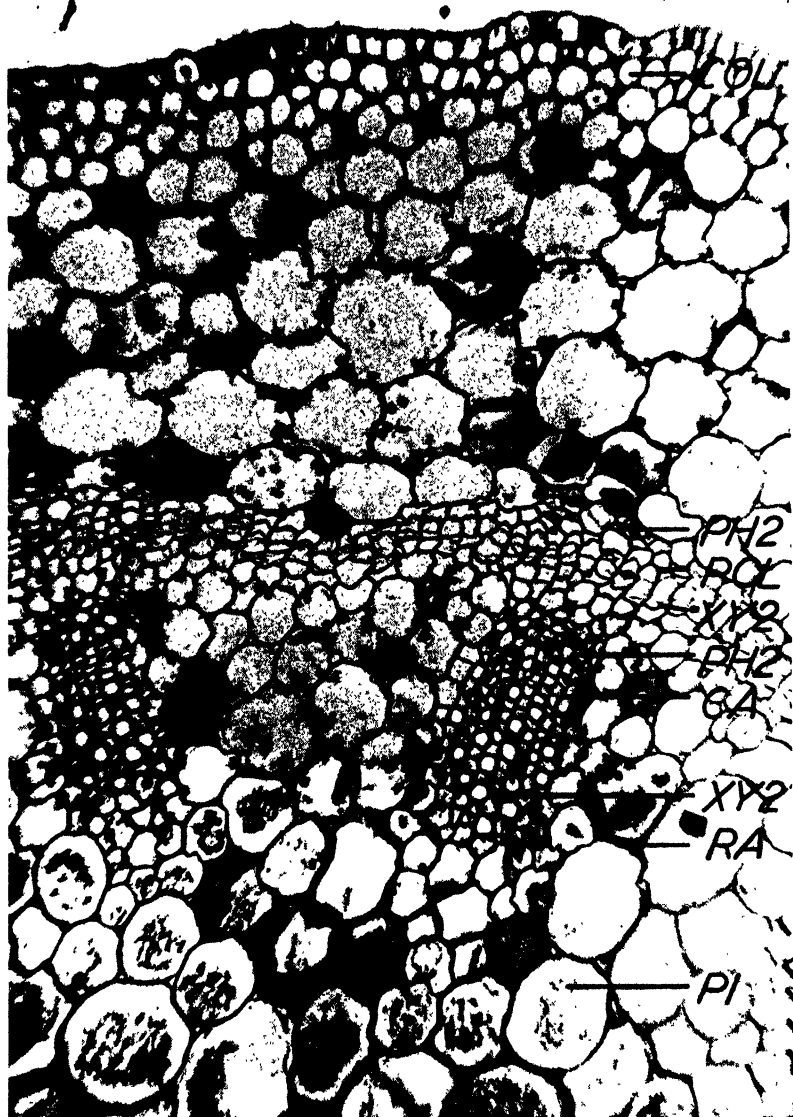


FIG. 11.—Transection of normal third internode at beginning of experiments, showing tissues and stage of development (*col*, collenchyma; *pcl*, pericycle; *ph*, secondary phloem; *xy*, secondary xylem; *ph*, secondary phloem of bundle; *ca*, cambium; *xy*, secondary xylem of bundle; *ra*, ray; *pi*, pith).



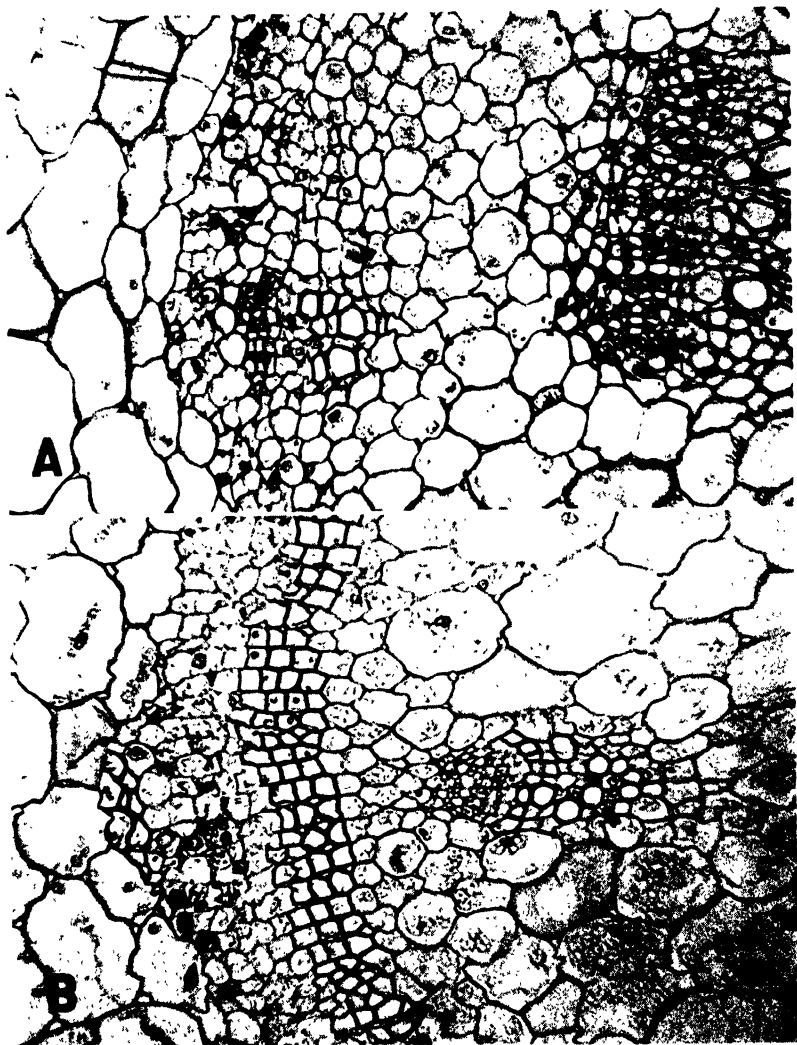


FIG. 12.—*A*: third internode 48 hours after lateral treatment, showing phloem of outer bundle and other derivatives of pericycle beginning to proliferate. *B*: eight hours after treatment; pericycle and its unthickened derivatives outside of thickened secondary xylem cells have become very active; tissues inside this ring not affected.

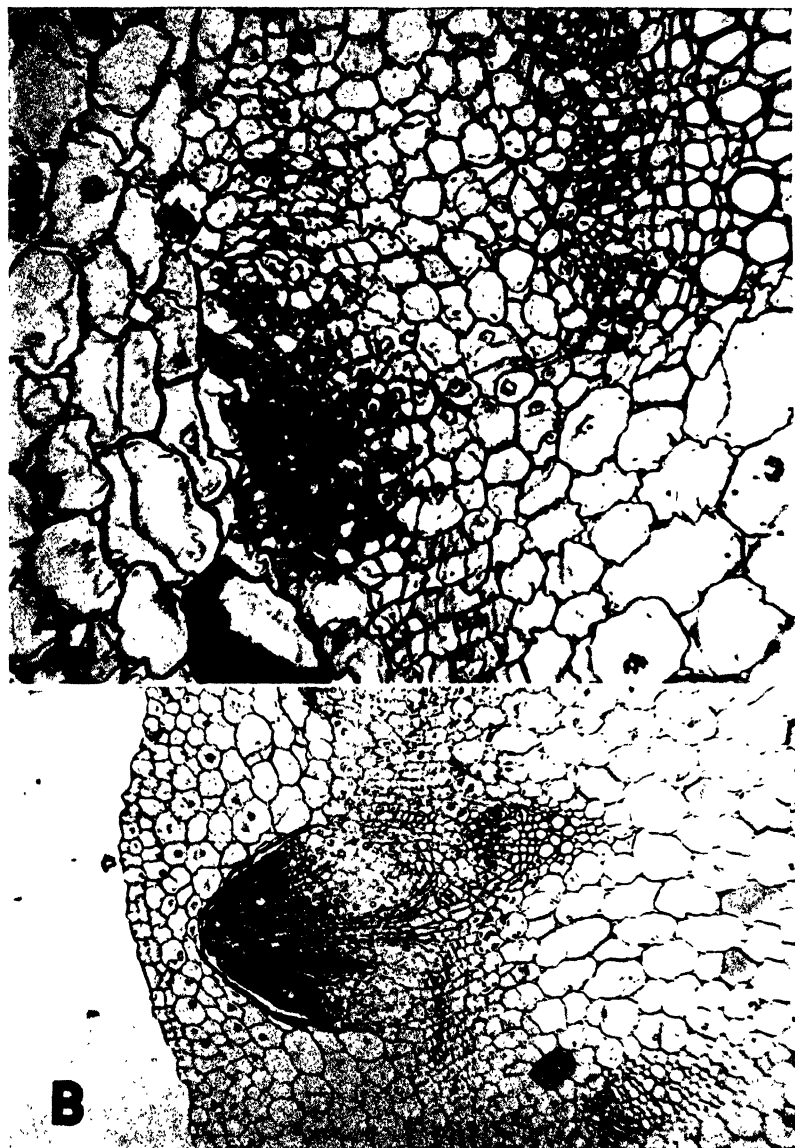


FIG. 13.—Third internodes in which the conjunctive tissue had not thickened at time of treatment. *A*: root primordium arising from pericycle and its derivatives; cells inward to primary bundle becoming involved. *B*: older root primordium, 216 hours after treatment, from which vascular tissue is differentiating back to primary bundle but also back to secondary bundle. Pericyclic tissue above bundle has proliferated to form a wide band; ray and cortex slightly active; most of epidermal cells have divided tangentially but with little enlargement.

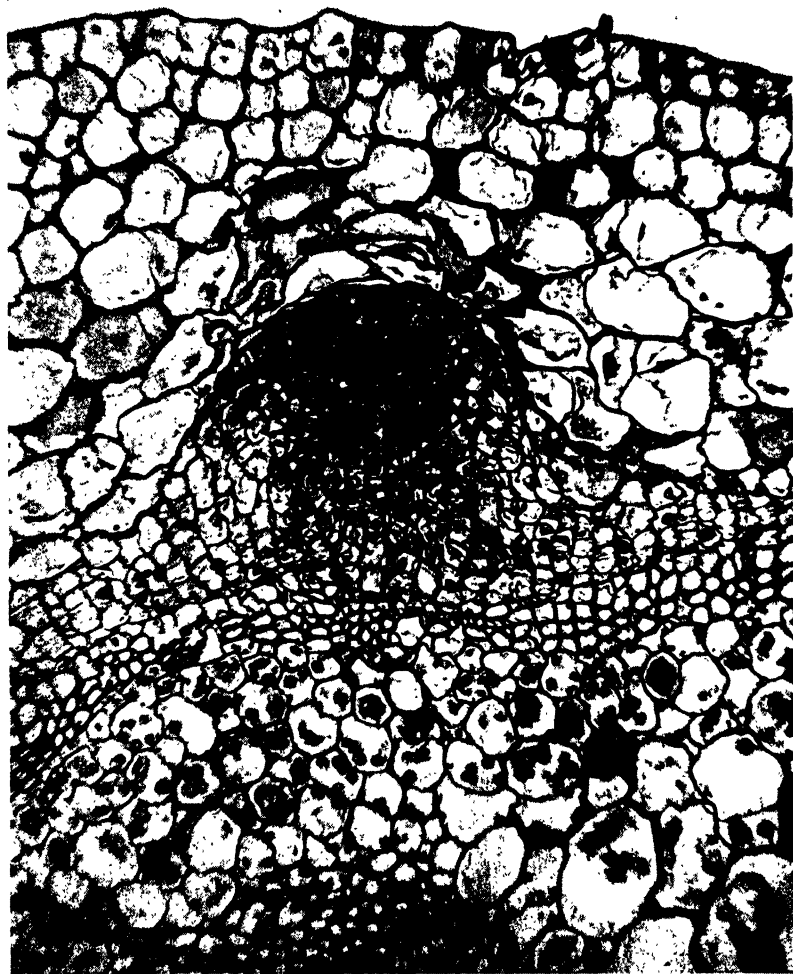


FIG. 14.—Internode 144 hours after treatment; primordium of root which has developed from tissue outside of band of thickened conjunctive tissue. Only epidermis, pericycle, and its derivatives are active.



FIG. 15.—Internode 216 hours after treatment; older root primordium which has developed from tissues outside of thickened band. Vascular tissue of root has differentiated back and tied up with xylem of secondary bundle formed from pericycle. No tissues inside thickened band are involved.

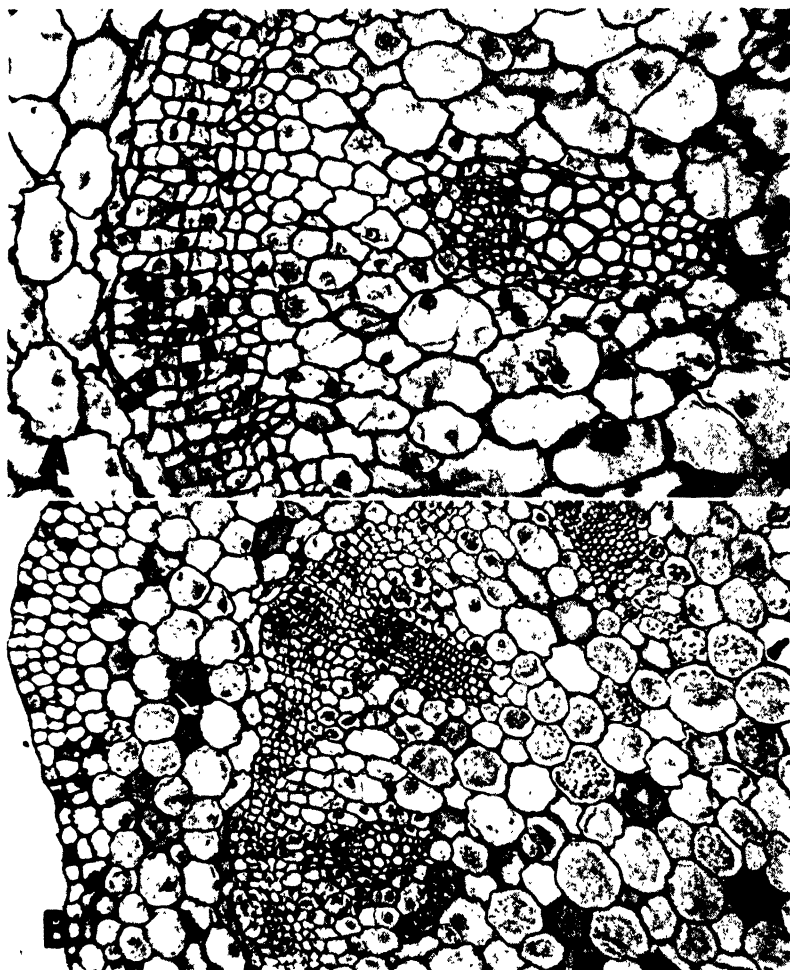


FIG. 16.—Decapitated third internodes apically treated. *A*: 80 hours after treatment pericyclic band has begun to proliferate. *B*: 110 hours after treatment. Phloem of primary bundles, derivatives of cambium, and ray tissue have also become active; root primordium developing in pericyclic zone; other tissues not affected noticeably.

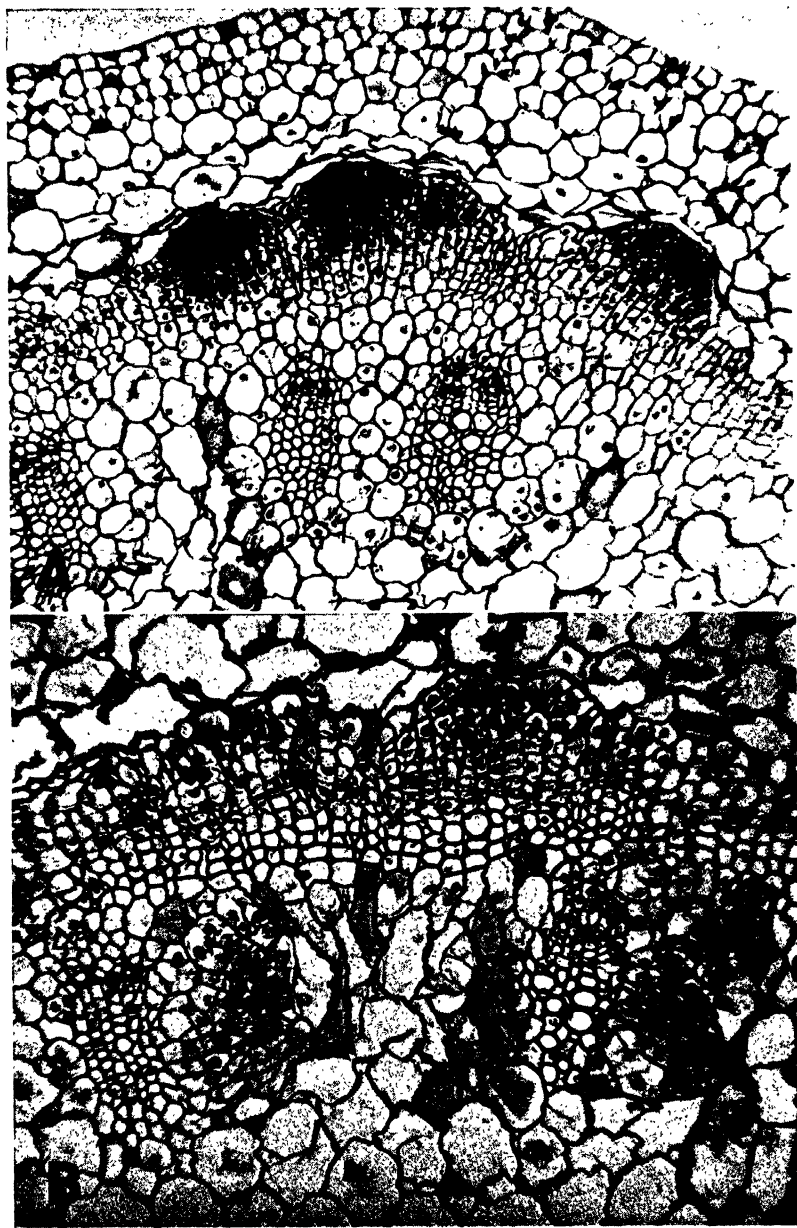


FIG. 17.—Apically treated third internodes. *A*: 144 hours after treatment. Most cells of pericyclic zone are meristematic; three root primordia have developed from this region. Pith cells adjacent to primary xylem of one bundle have divided repeatedly. *B*: 168 hours after treatment. Two root primordia have arisen from ray tissue adjacent to bundles and are growing sideways instead of outward. Pericyclic derivatives outward from thickened cell are very meristematic.

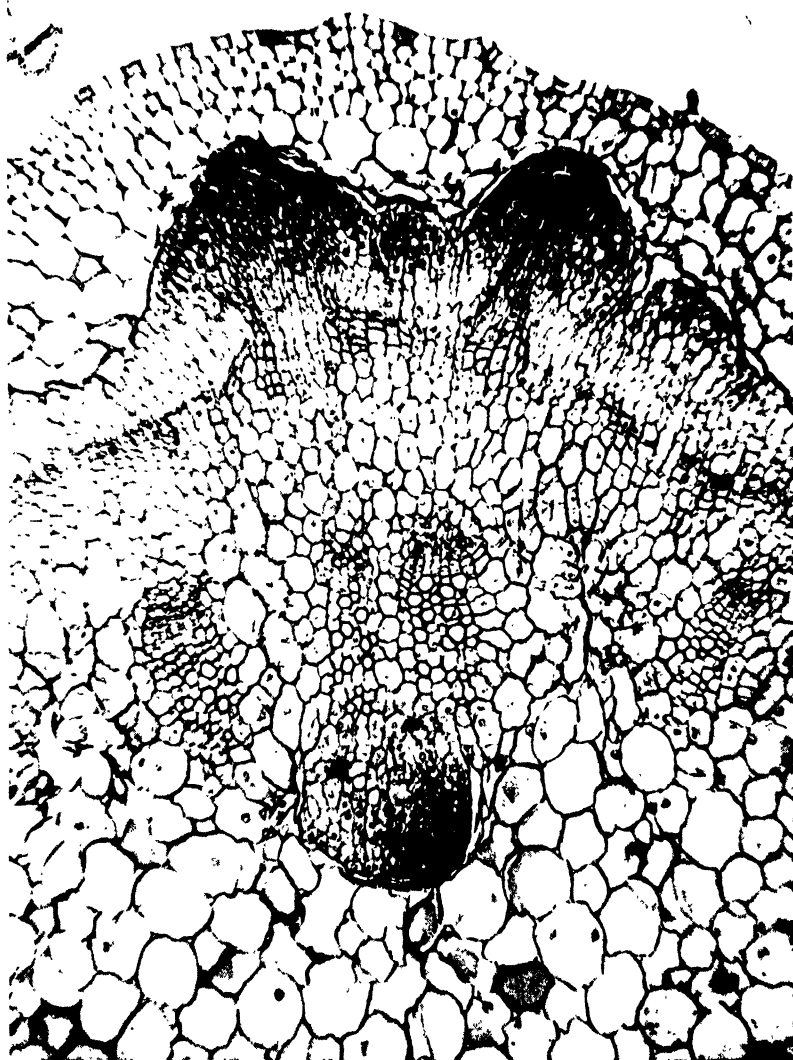


FIG. 18.—Third internode 240 hours after apical treatment. Root primordia developed from pith cells adjacent to primary xylem and growing inward. Xylem parenchyma cells have enlarged, pushing xylem elements apart. Other primordia growing outward from pericyclic zone. Vascular tissue is differentiating back to xylem of outer ring and also to primary bundles. Ray tissue highly meristematic; epidermis and cortex hardly affected at this level, which is about 1.25 mm. below cut surface.

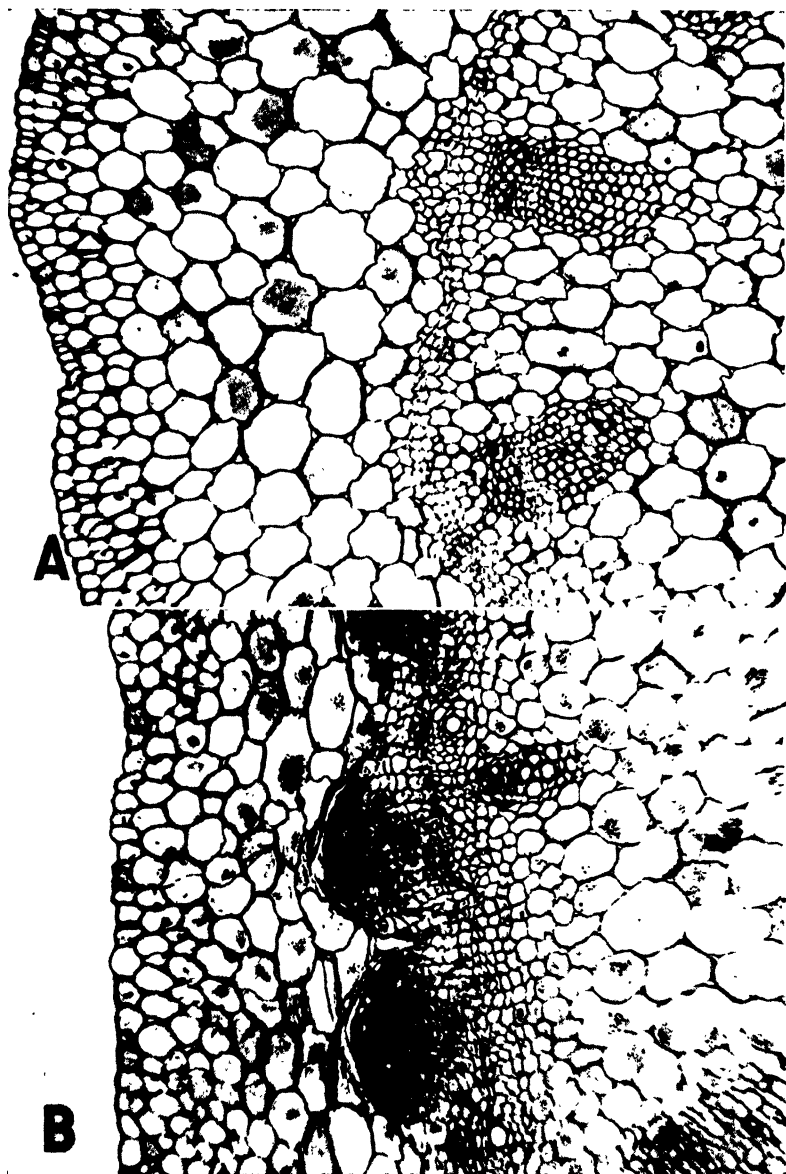


FIG. 19.—*A*: decapitated third internode treated apically with pure lanolin as control, 384 hours after treatment. Cells of epidermis and collenchyma slightly elongated radially; other tissues as in normal stem of this age. *B*: normal rooting; root primordia produced by stem cutting, untreated except for planting in sand; 144 hours after planting; about third or fourth internode.



internal to the ring of conjunctive cells. The cells of the phloem, rays, xylem parenchyma, and the derivatives of the fascicular cambium all proliferated much as in apically treated first internodes. The cells of the pith and of the ray tissue, however, showed even greater activity than did similar tissues in apically treated first internodes. In a number of cases root primordia were organized from proliferating ray cells, inward from thickened conjunctive cells. Such roots were prevented from growing outward by the layers of thickened conjunctive cells (fig. 17*B*).

Of more unusual occurrence was the development of root primordia from cells of the pith, adjacent to the protoxylem (fig. 18). The cells from which these roots developed are some distance from the nearest phloem (figs. 17*A*, 18). A number of such primordia were observed in several different stems.

In decapitated third internode controls which were treated apically with pure lanolin, the epidermal cells enlarged slightly. The cells of the collenchyma enlarged considerably but very few divided. Within the upper 750  $\mu$  the cortex and pith became active, the cells dividing in all planes. There was also a slight enlargement of the pith and cortex. Below 1 mm. from the treated surface the tissues appeared about as in an untreated stem (fig. 19*A*).

### Discussion

Stems of *Iresine* proved to be extremely sensitive to aqueous solutions of indoleacetic acid; treatments which in other herbaceous plants produce greatly increased rooting were highly toxic to them. Increased growth and cell division took place when the acid was supplied in dilute aqueous solution for a short time, or in lanolin paste from which it diffuses slowly.

The proliferation of cutenized epidermal cells with thickened walls in response to treatments appears to be of rather unusual occurrence, although CROOKS (2) has reported the formation of shoots from the epidermis of flax seedlings following wounding, and the enlargement of the epidermal cells of treated tomato and bean plants has been reported. Whether this response results from the presence of indoleacetic acid is questionable, since stems treated with pure lanolin react in the same way and to about the same degree. Perhaps the

results may be owing in part to the inhibition of gaseous exchange and respiration. Similarly, in stems treated with the lanolin mixture the cells of the collenchyma enlarged and divided, often losing all resemblance to collenchyma; but when treated with pure lanolin such cells enlarged greatly but very few divisions were observed.

The activity of the ray tissue and the phloem in the first internodes is similar to that reported for the epicotyls of beans, but *Iresine* differs sharply from the latter in the lack of pronounced activity of the endodermis, cortex, and pith. No large apical tumors which rise above the treated surfaces are formed in *Iresine*; such proliferation as does occur is limited largely to the first millimeter below the cut surface. While nuclear divisions follow in close succession in the proliferating cells, multinucleate cells as reported for the bean were not observed, although an occasional binucleate cell was found. The general degree of activity of *Iresine* resembles more closely that reported for the tomato than that of the bean. The production of lateral roots is similar in respect to origin, development, and behavior in beans, tomatoes, and the first internodes of *Iresine*. In the third internodes of the latter a different anatomical pattern as a result of secondary thickening is present at the time of treatment. Whereas in the first internodes at time of treatment only primary vascular bundles have been differentiated, in the third internode this series of primary bundles has become surrounded by a meristem from the pericycle and its derivatives. Thus a different anatomical pattern constituting quite a different distribution of cells of greater or lesser degrees of differentiation or maturation results in a somewhat different series of responses to treatment with the lanolin mixture. In laterally treated third internodes the only tissues to respond markedly are those which lie external to the thickened conjunctive tissue. That the indoleacetic acid does not reach the tissues inside this band in sufficiently great concentration to cause them to react seems possible when the behavior of apically treated third internodes is considered. Something more than the concentration of indoleacetic acid must be involved, however, since the quantity which stimulates the pericycle and its derivatives to become highly meristematic must pass through the cortical cells; but they do not respond beyond a comparatively limited enlargement. The sup-

position that the band of thickened conjunctive tissue hinders or prevents the passage of indoleacetic acid beyond its inner limits—and as a result there is an accumulation of the indoleacetic acid in the pericycle and its derivatives which have not become thickened—may be a plausible explanation for the great activity of these tissues, but such an explanation could not account for the varied responses of different tissues when apical treatments to the cut surfaces are made. There appear to be some inherent differences in the capacities of cells of different tissues to respond.

In sharp contrast to the responses to lateral treatments, the tissues within the cylinder of thick walled conjunctive cells became very active following apical treatments. Not only did the cells of these tissues enlarge and divide but some of them gave rise to root primordia. The most noteworthy example of this was the organization of root primordia from cells of the pith. The initiation of roots which grew inwardly following treatment with the lanolin mixture has been reported previously for tomato (1). The internal roots in tomato are reported as arising from the internal phloem. It has been suggested that growth-promoting substances are transported in the phloem, and this may be true in the case of tomato. In *Iresine*, however, the internal roots developed from pith cells located near the protoxylem and at some distance from the nearest phloem. It is unlikely that the cells which initiated these roots were stimulated directly by indoleacetic acid which was transported in the phloem. It is more feasible to assume that they were stimulated by indoleacetic acid which passed down the adjacent xylem elements. This tends to support the suggestion of HAMNER and KRAUS (5) that “apparently some of the indoleacetic acid may travel in vessels in the xylem.”

In stems of the age used in this investigation the fascicular cambium is relatively active. There is, however, no marked increase in the activity of this cambium following treatment with indoleacetic acid. It appeared to maintain about the same relative rate of division as in untreated stems. This is in contrast to the condition reported for *Helianthus* (9), for *Coleus* (4), and for bean (6). It is also noteworthy that in laterally treated stems the collenchyma

cells with heavily thickened areas become more markedly meristematic than do the parenchymatous cells of the cortex.

A rational explanation for the variations in reactivity of different tissues of different plants must await further detailed information on the movement of the various growth substances in plant tissues and the conditions under which their effects on the various cell constituents become manifest. The relative degree of maturation of the tissues and the cells, the external environment, and the variations in the so-called correlative factors at the time of treatment must all play a part in the rate and extent of the responses exhibited.

### Summary

1. The rooting of stem cuttings of *Iresine lindenii* treated with weak aqueous solutions of indoleacetic acid for a short time was hastened and the number of roots increased.

2. First and third internodes were each treated with 3% indoleacetic acid in lanolin applied laterally as a ring or apically to decapitated stems. Similar internodes were treated with pure lanolin as controls.

3. The gross responses of the plants were observed over a period of two to four weeks. Tumors were formed and numerous adventitious roots developed in the treated areas.

4. In the older internodes an extrafascicular cambium derived from the pericycle gives rise to a band of conjunctive tissue and secondary vascular bundles. Differences in the responses of first and third internodes may be correlated with this structural feature.

5. Histological studies showed that all living tissues of the stem react to some extent to treatments with indoleacetic acid. The cells of the pericycle and its derivatives, rays, and phloem were the most generally responsive, although the epidermis and collenchyma became very active in laterally treated stems. Neither the cambium nor the endodermis was markedly stimulated.

6. Lateral roots developed from the tissue of the ray, phloem, and pericycle in first internodes. In third internodes roots external to the conjunctive tissue developed from the pericycle and its derivatives and from the phloem of secondary bundles. Such roots as

were formed internal to the conjunctive tissue developed from the ray tissue and from the pith.

7. The responses of treated *Iresine* stems were compared with those of other plants and some of the implications are discussed.

The writer is indebted to Dr. E. J. KRAUS and Dr. C. A. SHULL for suggesting this problem and for their very generous help during the progress of the investigation.

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# LIFE HISTORY OF PELAGOPHYCUS PORRA

CHARLES C. HERBST AND GEORGE R. JOHNSTONE

(WITH TEN FIGURES)

## Introduction

The evidence (12, 13, 18, 19, 26) in support of the view that antithetic alternation of generations is generally present throughout the order Laminariales has been increasing during the last two decades. The present investigation of the life history of *Pelagophycus porra* (Leman) Setchell extends the evidence in support of this view. It is also desired to emphasize the method of culture. HARRIES (8) seems to be the only one who has measured the light intensity factor.

TAXONOMIC RELATIONS.—According to SETCHELL (24), the first botanical description of this species was given by LEMAN, who named it *Laminaria porra*. The type specimen, obtained by Dr. GUSTAV EISEN, was described by ARESCHOUG (4) as *Nereocystis gigantea*. ARESCHOUG (5) later separated it from *N. luetkeana*, making it the type of a new genus, *Pelagophycus*. SETCHELL's (24) observations as to the unilateral splitting of the blade and the consequent close relation to *Macrocystis* caused him to retain the genus, *Pelagophycus*, and to associate it with the Macrocystieae under the tribe Lessonieae. The description of the species and its classification are given by SETCHELL and GARDNER (25) in a later publication.

CULTURE METHODS.—The important factors to consider in the culture of marine algae are the mineral requirements, the reaction of the various mediums, temperature, and light intensity. It has been suggested by HARVEY (10) that sea water contains everything necessary for the life of marine organisms with the exception of phosphates and nitrates, which are present only in minute quantities. Sometimes their lack limits plant growth. HARVEY thinks that the greatest amounts of these salts occur during the winter months; that they are almost completely used up during the summer, but toward the end of October they attain their maxima in the surface water, being

replenished from decaying plant and animal bodies which sink to the bottom layers. The seasonal variation of the nitrate and phosphate content of sea water near the shore has not yet been accurately estimated, but presumably it follows that of the open sea, although with greater fluctuation because of contamination. Data presented by SCHILLER (22) show that a constant slightly alkaline reaction is essential when using culture mediums of sea water plus nutrients.

ARBER (3) found that additions of nutrient solutions containing sodium nitrate, potassium nitrate, and sodium hydrogen phosphate to sea water were satisfactory in promoting the growth of the gametophytes and the young sporophytes, and KILLIAN (11) later used a similar nutrient solution in the culture of various species of *Laminaria*. DREW (7) used glass jars of 600 cc. capacity which were thoroughly washed and filled with sea water to which the nutrient solution had been added. The cultures were then inoculated with small pieces of mature reproductive areas which had been gently brushed and washed in sterilized sea water. WILLIAMS (27) and SAUVAGEAU (18, 21) have grown plantules of *Laminaria* in culture using similar methods. KYLIN (12) made cultures of *L. digitata* in small glass dishes containing 30-40 cc. of sea water. Portions of a mature thallus bearing mature sporangia were left in the water for a few days to permit the escape of zoospores. The fragments of sori were then removed to reduce bacterial activity. He noticed that in sea water in which 2 per cent sodium nitrate had been added there was an abundance of small sporophytes; that the chromatophores were a strong brown color and larger than the ones in sea water alone. Macroscopically there was a significant difference between the two groups of cultures, for in the nitrate cultures the bottoms of the dishes were covered with a vigorous, deep brown layer of vegetation; in those without nitrogen, a relatively thin yellow brown layer. While working with *Chorda filum*, KYLIN (13) found that it was only in the cultures to which he had added a small quantity of calcium phosphate plus the 2 per cent sodium nitrate that fertile *Chorda* gametophytes were obtained. In the cultures without phosphate the cells of the gametophytes were distended like balloons, and their chromatophores were relatively small and pale yellow brown in color; after five or six months the gametophytes began to die with-

out having become fertile. PRINTZ (17), working on the Alariae, collated KYLIN's culture proceedings. MYERS (14, 15), ANGST (1, 2), HARTGE (9), and MCKAY (16) followed the general procedure as outlined by KYLIN. HARRIES (8), using culture methods to study some of the factors influencing the development of the gametophytes and the early stages of the sporophytes of *Laminaria digitata*, found that zoospores, provided with sea water alone or with sea water plus minute quantities of nutrient material, produced germ tubes, passed into a temporary resting stage, and remained in that condition or increased in size only when the nutrient was increased. The antheridia and oogonia formed only within a restricted range of nitrate-phosphate concentrations. When the initial water was not removed no fertile megagametophytes or microgametophytes were formed.

As to the temperature requirements of the germinating zoospores, SAUVAGEAU (18, 19, 20) and KYLIN (12) merely mentioned placing them in a north window in a cool place. SCHREIBER (23) found that lowering the temperature of the water to 4°–6° C. induced the production of gametes, whereas raising the temperature to 18° C. suppressed production, and that gametic propagation is dependent upon lower temperatures. Culture experiments as well as observations at various stations by SCHREIBER have shown that the vegetative tendency to grow is not retarded by higher temperatures. HARTGE (9) cultured *Nereocystis* at an average temperature of 16° C. maintained by a continuous flow of sea water among the culture dishes in a water bath. MYERS (14, 15) and MCKAY (16) used a special cooling cabinet and found that the temperature range of 12°–16° C. proved to be the most satisfactory for growth and reproduction, but noted that gametophytes developed at 4° C. and that oogonia and antheridia were first observed about a week after the cultures had been moved to a temperature of 12° C. Plants growing at a temperature of 12°–20° C. grew normally but failed to reproduce, owing to the higher temperature.

HARRIES (8) was the first to perform controlled light experiments with species of Phaeophyceae. Working with *Laminaria digitata* and *L. saccharina*, the gametophytes were subjected to different portions of the visible spectrum. The results showed that the blue rays of



the spectrum allow for complete development but when the red portion only is available, growth and antheridial formation are retarded and oogonial formation is definitely inhibited. SAUVAGEAU (18, 20), KYLIN (12), MYERS (14, 15), HARTGE (9), and MCKAY (16) recommend placing the cultures in northern exposures.

### Material and methods

**COLLECTION.**—Regular bi-monthly collections were made from October 1934 to April 1935 by means of dredging operations from the Marine Station launch in kelp beds located in water 7–12 fathoms deep, 5–7 miles due south from the Breakwater Lighthouse, San Pedro Harbor, California, which is located at latitude  $33^{\circ}43'12''$  north and longitude  $118^{\circ}16'20''$  west.<sup>1</sup>

The plants were measured on the launch and the mature sori removed. These were placed in glass jars of sea water or wrapped in moist paper and placed in a wet sack and brought to the laboratory. Sea water for later culture purposes was obtained at a depth of several meters to avoid contamination from the surface plankton. Temperature readings of the sea water at 1 meter depth were made on each trip.

**METHOD OF CULTURE.**—In the laboratory, pieces of the fruiting sporophylls were washed thoroughly in sterilized sea water, using a stiff brush in order to remove, as nearly as possible, all foreign matter which adhered to the gelatinous surfaces, as difficulty was always experienced in setting up cultures that were free from contamination. The culture solution used in the present investigation was essentially the same as that originally recommended by KYLIN (12), and consisted of filtered sea water to which were added traces of calcium phosphate and sodium nitrate. In order to obtain the best results and avoid contamination, the culture solution was made up of sea water sterilized in the autoclave at 15 pounds pressure for 30 minutes and allowed to cool. To this was added sodium nitrate in the ratio of 1 gm. per liter, a small crystal of potassium hydrogen phosphate, and a trace of calcium chloride. Chemically pure salts were used. It was found best to sterilize the water just before using,

<sup>1</sup> Map of Los Angeles and Long Beach Harbors. Department of Commerce, U.S. Coast and Geodetic Survey. 1932.

as sea water allowed to remain in glass jars for any length of time forms a precipitate. Air was added to the water after sterilization by pouring the water back and forth several times from a height of 20–30 cm. All dishes, glasses, and glass slides were sterilized. A specially constructed glass-inclosed culture cabinet situated on the north side of the laboratory, where it was never exposed to direct sunlight, was used for all the culture dishes. The temperature was controlled within 2° of that desired. Two types of culture dishes were employed. Glass culture jars of 750 cc. capacity were tried at first, but later discarded for glass tumblers of 250 cc. capacity as the latter were found to be more convenient in making the necessary weekly changes of the nutrient solution. It was also found advisable to start the culture in smaller glasses so that in case of contamination one or several of them might be thrown out without destroying all the cultures from a particular collection. Microscope slides were placed in the culture dishes, on which the plants became attached. Ten to twelve fragments of the sori about 1 cm. square were placed in each dish and allowed to stand from 12 to 24 hours before the sporophyll material was removed and a new nutrient solution added.

### Data on technique

EFFECT OF TEMPERATURE.—Various degrees of low temperature approximating that of sea water under natural conditions have been used (table 1), and it was found that a range of from 8° to 12° C.

TABLE 1  
TEMPERATURES MAINTAINED DURING  
GERMINATION

DATE	DEGREES CENTIGRADE
11/25/34 to 1/1/35	8 33 to 10.00
1/1/35 to 2/8/35	14.44 to 15.56
2/8/35 to 4/15/35	11.11 to 12.22

was best for gametophyte development and growth. At temperatures of 14.44°–15.56° C. the chromatophores became pale. Data in tables 1 and 2 show that the optimum range of temperature in the culture cabinet was below that of the average surface temperatures of the sea water taken during the collecting expeditions, and also lower

than the average monthly surface temperatures at points along the shore. This lower optimum temperature no doubt approximates that

TABLE 2  
COMPARISON OF SURFACE TEMPERATURE IN ° C.

MONTH	COLLECTING EXPEDITIONS (1934-1935)	LA JOLLA * 1926-1934	BALBOA PIER * 1926-1934
October.....	16.25	17.22	17.43
November.....	15.30	15.42	16.24
December.....	15.00	13.91	15.12
January.....	15.00	13.01	14.09
February.....	14.25	12.60	14.13
March.....	13.50	13.46	14.52
April.....	13.75	14.90	13.35

\* Mimeographed sheets, Scripps Institution of Oceanography, La Jolla, California.

TABLE 3  
MEASUREMENTS OF REFLECTED LIGHT IN CULTURE CABINET

LOCATION	MAXIMUM LIGHT	RED RAYS		BLUE RAYS	
	Foot candles	Foot candles	Per cent	Foot candles	Per cent
Without cheesecloth screen					
Front of cabinet.....	207.3	5.7	2.7	16.4	7.9
Back of cabinet.....	143.5	4.7	3.2	13.4	9.3
Shelf of cabinet.....	170.5	6.3	3.6	14.0	8.2
Average.....	173.7	5.9	3.1	14.6	8.4
With cheesecloth screen					
Front of cabinet.....	82.2	2.9	3.0	6.5	8.0
Back of cabinet.....	63.0	2.0	3.0	5.2	8.0
Shelf of cabinet.....	80.2	2.3	2.8	5.8	7.0
Average.....	74.8	2.4	2.9	5.8	7.7

at the lower levels in the ocean where the plants grow under natural conditions.

**IMPORTANCE OF LIGHT.**—In view of the importance of light, a number of measurements were made to determine the kind and intensity of light entering the culture cabinet containing cultures of marine algae. A photometer as described by CUSHMAN (6) was employed. When used with a color filter before the face of the cell, the instrument measures the intensity of the light passed by the filter.

A comparison was made of the intensities of the red and blue portions of the spectrum together with the maximum intensity of diffuse light as received on the face of the photoelectric cell when placed in the culture cabinet.

The results (table 3) show that the cabinet received an average of 173.7 foot candles of natural reflected light, 8.4 per cent of that amount being in the blue end of the spectrum and 3.1 per cent in the red region. To reduce the amount of indirect light in the cabinet a cheesecloth screen was used, and the results showing the amount of light cut off are given (table 3) for comparison.

### Morphological results

The sporophytic generation of *Pelagophycus porra*, the so-called bull kelp or elk kelp, includes a large conspicuous plant of a monotypic genus confined to a relatively small area on the Pacific coast of North America, and at the present time is known to occur from the vicinity of Point Conception, California, to some locality on the coast of Lower California, Mexico, where its southern limit is not well defined.

Recent data (table 4) taken from fresh specimens *in situ* based on an average measurement of twenty-five plants have increased the dimensions for all parts over those of SETCHELL (24). As many as twelve to fourteen blades have been found on mature specimens, with the first branch nearly always dividing once dichotomously, while many times the terminal and final branching were found to be dichotomous. The surface of the blade is coarsely and transversely rugose and is nearly always found with a dense covering of *Obelia* and *Bryozoa*. The sporangia are found on macroscopic sporophylls, borne in extended sori at the tips of the fronds. In the younger sporophylls the sori are entire, but in the older ones the ends become frayed and large patches of disintegrating sori are found.

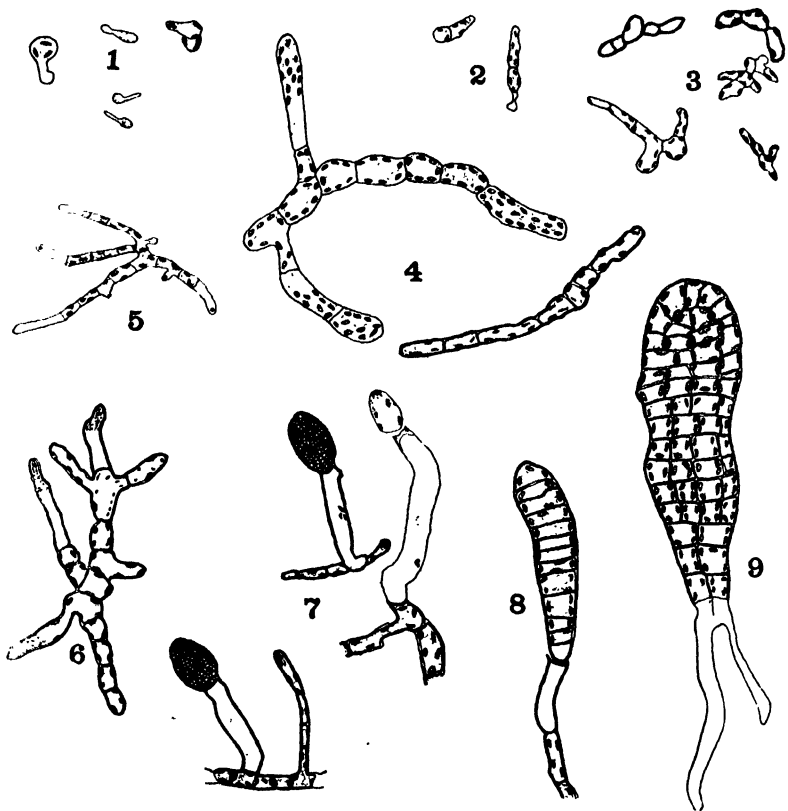
DEVELOPMENT OF SPORANGIA.—The sporangia are borne in irregular, dark colored sori which occur on both surfaces of the sporophylls. The sorus is made up of a palisade-like layer of sterile unicellular paraphyses having terminal hyaline appendages and fertile sporangia borne side by side. Both of these organs originate from the superficial cells of the cortex of the sporophylls. As the sporangium matures the cytoplasm draws away from the wall, leaving the approximate thirty-two spores crowded together. In addition to a small amount of cytoplasm, each spore contains one chromatophore. As the spores develop, the wall at the apex of the sporangium gradually becomes thickened, forming a caplike structure. As the

TABLE 4  
COMPARISON OF PLANT MEASUREMENTS

STRUCTURE	SETCHELL 1925	HERBST AND JOHNSTONE 1934-1935
Length of stipe.....	6-7 m.	6-10 m.
Holdfast .....	1 5 dm. diameter	2 3-8 5 dm. diameter
Apophysis. ....	80 cm. long	60-90 cm. long
Width of blade.....	20-45 cm.	30-76 cm.
Length of blade.....	4 0-5.5 m.	1 9-6.0 m.
Length of arm.....	1.3-1 6 m.	1.4-2.0 m.
Outside diameter of pneumatocyst.....	12-20 cm.	9.0-25 0 cm.

time approaches for the mature spores to leave the sporangium, the more nearly terminal ones are seen to line up toward the apex, gradually emerge and float away. While watching this process on a slide, three spores have been seen to emerge in 45 minutes. Contrary to the findings of other workers, in the Laminariaceae the spores do not seem to possess cilia or to be motile. Reference is made in the literature cited that the zoospores possess two lateral cilia of different lengths and that the zoospores swim rapidly away upon being released from the sporangium. Although various staining techniques were employed, no cilia were observed. Continued observations of material at different times of day and night failed to disclose any motility of the spores. The movement in all cases was slight, but the fact that they attached themselves to the slides in the culture dishes and germinated in the normal way would indicate that they have sufficient buoyancy to float to a place of attachment.

The spore is spherical, or occasionally ovoid, measuring  $3.5-4\ \mu$  in diameter. When placed in the artificial culture solution, the spores float for 8-24 hours and then attach themselves to some sur-



FIGS. 1-9.—Camera lucida drawings from living material: fig. 1, stages in spore germination; fig. 2, two-celled gametophytes; fig. 3, gametophytes showing various stages of development; fig. 4, 25-day old gametophytes showing male (slender) and female filaments; fig. 5, microgametophyte forming antheridia; fig. 6, megagametophyte forming young oogonia; fig. 7, megagametophytes with mature eggs and empty oogonium; fig. 8, sporophyte showing transverse division of cells; fig. 9, 140-day old sporophyte showing rhizoids.

face, begin to enlarge, finally reach a diameter of  $10-12\ \mu$ , and secrete a distinct wall (fig. 1). Germination begins and continues for several days, the spores producing germ tubes. The chromatophore elongates and passes into the tube, usually dividing during the process of migration. When the plants are about one week old the

first cross walls appear in a few of the plantules (fig. 2), and several days later the majority of the germinating spores form the first wall across the germ tube.

**GAMETOPHYTES.**—The plantules remain in a temporary resting stage for a few days to several weeks, the increase in size being very gradual. Within 16 days some of the gametophytes form monosiphonous filaments 3–5 cells in length (fig. 3), and by the end of the 20–25th day the plants are 8–14 cells in length. The rate of growth then increases and is manifested by elongation of the plants and differentiation of the microgametophytes and megagametophytes. The microgametophytes are smaller, multicellular, and branched, the branches being short and compact or thinner and more elongated. Their growth at first appears to be more rapid than that of the megagametophyte, and the chromatophores are paler, so that the sex of the gametophytes can easily be distinguished by the difference in pigmentation as well as by the smaller diameter of the male (fig. 4). Any cell can function as an antheridium, the end cells being first converted. As the process of antheridial formation proceeds and the sperms are discharged, the microgametophyte becomes gradually invisible.

It is possible to distinguish between the two kinds of plants within 30 days. The larger immature megagametophyte contains 10–14 cells measuring 8–10  $\mu$  in diameter, while the immature microgametophyte is more profuse in growth, consisting of 12–17 cells, 6–8  $\mu$  in diameter. Under favorable conditions the gametophytes have been found to have reached maturity and to have produced gametes in about 48 days after the germination of the spores. Mature microgametophytes have individual cells averaging 12–15  $\mu$  in length by 10–14  $\mu$  in diameter. Mature megagametophytes have individual cells averaging 30–35  $\mu$  in length by 16–18  $\mu$  in diameter. Many mature gametophytes have been found measuring 105  $\mu$  in length.

**FORMATION OF ANTHERIDIUM.**—The mature microgametophyte bears slender, irregular branched filaments. The first reproductive structures appear in approximately 40–50 days. The end cells of these filaments or of their branches may become antheridia, or antheridia may develop as one-celled outgrowths from the sides of the branching filaments (fig. 5). The chloroplasts cannot be dis-

tinguished in them so clearly as in the other cells, and they are filled with a rather dense cytoplasm. The process begins in the end cells, which become much paler; the wall at the apex of the cell swells greatly and the single antherozoid breaks out and moves away. Ultimately most of the cells of the gametophyte become emptied of their contents. One of the first signs of the approaching maturity of a culture is the appearance in it of a few empty terminal cells. Soon after the antheridium is formed, the contents round up and pull away from the wall. The entire contents of the antheridium are used in the formation of a single male gamete. The chromatophore gradually grows pale and less distinct in form, until by the time the antherozoid is discharged, it may appear as an indefinite brownish green mass at one side of the cell. MCKAY (16) describes a similar situation in regard to the microgametes of *Pterygophora californica*, where the gametes are a brownish green mass or almost colorless. The spherical antherozoid is about  $2.2-3.3 \mu$  in diameter, having the general appearance of a minute spore. Since the gametophytes are microscopic in size and the process of liberation of the antherozoids must be watched through a microscope, it is only by chance that one is able to observe them escape. The situation is made still more complex by the fact that often, especially in old cultures, small protozoans may be present which are very similar to the antherozoids in size, shape, and position. In order to make certain of the identity of the antherozoids, one must actually see them liberated from an antheridium and follow them in their movements. Their escape was not observed; whether the forms which emerged were free-swimming is still to be determined. The evidence that there were sperms was the presence of antheridial structures in the cultures. Some contained single small bodies while others were empty, apparently having been abandoned by the sperms. Recorded observations on other genera led also to this conclusion.

FORMATION OF OOGONIUM AND EGG.—Cultures 50 days old, containing mature megagametophytes with erect branches, are commonly found bearing large apical cells which are conspicuous on account of their dense contents (fig. 6). These cells elongate to about twice the length of the normal vegetative cells as the wall at the apex becomes swollen.



The gametophytes increase in diameter, and are a richer brown in color owing to the increased number of chromatophores. The walls of the cells have a somewhat heavier appearance, with the apical cells of many branches several shades darker than the rest of the filament. The length of the cell is much augmented and its apex becomes decidedly swollen. This makes the oogonial structures strikingly noticeable. The oogonium contains, in addition to the egg, a small amount of cytoplasm and a few chromatophores. The unfertilized egg is a spherical or ovoid body, surrounded by a delicate plasma membrane. It contains many chromatophores, which are distributed irregularly throughout the cytoplasm (fig. 10). At the apex of the oogonium the thickened wall splits, developing a beak through which the egg emerges (fig. 7). The egg rounds up as it emerges from the oogonium and usually remains in position at the tip, where it is fertilized. Although the egg usually remains at the mouth of the oogonium, it has been seen displaced and developing entirely away from the megagametophyte. There can be no doubt that here is a differentiated oogonium, and that the single egg produced by it is fertilized after emergence. While most of the young sporophytes remain attached to the empty oogonium, many become detached and float away. Under natural conditions this probably occurs far more frequently.

**FERTILIZATION.**—The sexual cells are liberated one or few at a time, with long intervals between. It is evident that the chances are very much against an observer being fortunate enough to see these stages at the critical time. Even continuous observation would not suffice to guarantee success, for fertilization may be taking place on a slide while it is being examined under the microscope, yet owing to the smallness of the microgametes the process may be missed completely. The cultures, especially if they are old, often have numbers of monads similar in size and shape to Phaeophycean bodies; this makes it very difficult to be certain of the identity of Pelagophycean antherozoids without having witnessed their liberation. In one case, only, a sperm was observed lying close to the tip of the egg. In the hour or more that the movement was watched the sperm would move from place to place over the membrane of the egg. The egg at times would swing away from the apex of the oogonium but always

returned to the original position. When movement had ceased, the antherozoid was about  $6\mu$  inside the membrane of the egg. Immediately after fertilization, the zygotes form a wall about themselves and begin to elongate.

DEVELOPMENT OF YOUNG SPOROPHYTE.—The zygote elongates and divides, producing two cells of unequal size. Cell division con-

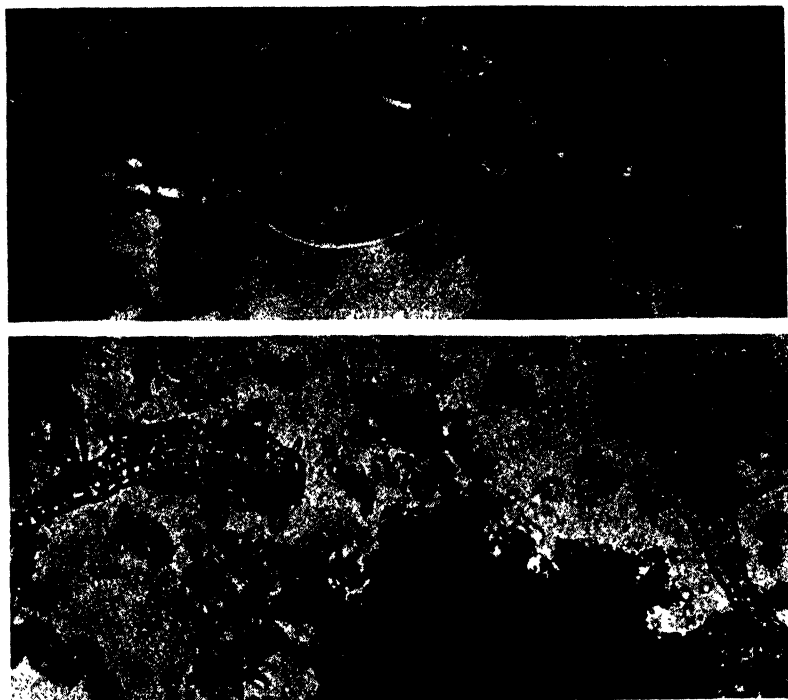


FIG. 10.—Top, egg from megagametophyte showing chromatophores and cell wall. Bottom, sporophytes 148 days old showing rhizoids developing from basal cell.

tinues in one plane only, resulting in a short thick monosiphonous filament of 6–12 cells (fig. 8). The cells in the apical region are the first to divide by a longitudinal septum, and are followed rapidly by the other cells of the filament, with the exception of the basal cell which begins to elongate. The sporophyte is not attached to the oogonium by the basal cell but is held in position only by the gelatinous matrix which surrounds the egg; in fact, sporophytes were ob-

served to swing about and were often seen floating free after the slides had been removed several times for observation. Plants 8-12 cells in length showed no indication of rhizoid formation; however, a number of that size were observed to have an elongated basal cell, and in a few cases it was septate. A month after the formation of the young sporophytes, rhizoids were observed growing as protuberances from the basal cells (fig. 9). The rhizoids are unbranched, contain no chromatophores, and have no cross walls (fig. 10).

### Summary

1. The development of the gametophytic and sporophytic generations of *Pelagophycus porra* was followed under the microscope by the frequent examination of living material from cultures.

2. The culture cabinet received 173.7 foot candles of natural reflected light as the average of several readings taken from 11 A.M. to 1 P.M. during January; 8.4 per cent of that amount was in the blue end of the spectrum and 3.1 per cent in the red region.

3. Alternation of dissimilar generations occurs in the life history. The massive macroscopic sporophyte alternates with the microscopic gametophytic generation.

4. The spores are morphologically homosporous but physiologically different, non-motile, and arise from unilocular sporangia. No cilia were observed, although various staining techniques were employed.

5. The sporangia develop spores which are identical in appearance with one another, but upon germination become independent microgametophytes or megagametophytes of microscopic size.

6. After the formation of the germ tube, the gametophytes may give rise to single filaments, or to two filaments growing in opposite directions.

7. Microgametophytes are smaller than the female plants and produce antheridia with single sperms, terminally or at the tips of lateral branches, in contrast to the clustered antheridia described by SAUVAGEAU and HARTGE. No sperms were observed to leave the antheridia, but several were observed inbedded in the matrices of the eggs.

8. Germinating spores produce 9- to 14-celled megagametophytes.

This observation agrees with the findings of MCKAY and HARTGE, but disagrees with those of SAUVAGEAU and KYLIN, who report having observed one- to few-celled filaments.

9. The megagametophytes are larger, more branched than the male, and produce a single oogonium at the tip of a branch. The egg is extruded and is apparently soon fertilized. Following fertilization the zygote forms a wall and the development of the sporophyte begins.

10. The young sporophytes are monostromatic, cell division taking place at first in one plane only. The apical cell divides by a longitudinal septum and is followed rapidly by division in the same plane by the other cells of the filament, with the exception of the basal cell which begins to elongate. Rhizoids were observed growing from the basal cell of sporophytes 140 days old.

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# HISTOLOGICAL COMPARISON OF FRUITS DEVELOPING PARTHENOCARPICALLY AND FOLLOWING POLLINATION<sup>1</sup>

F. E. GARDNER AND E. J. KRAUS

(WITH SEVENTEEN FIGURES)

An earlier issue of this journal (2) gave an account of parthenocarpny induced by spraying the open blossoms of the American holly, *Ilex opaca*, with dilute aqueous solutions of several growth substances, including indoleacetic acid. It was pointed out that the development and external appearance of the parthenocarpic fruits were quite like the fruits set following pollination. Even at maturity it has not been possible to distinguish between normal and parthenocarpic fruits except by cutting open the seeds which in the latter case were invariably devoid of embryos. GUSTAFSON (3) has also given a list of fruits whose development has been induced to a greater or lesser degree by growth promoting substances, but has not recorded histological details.

In view of the marked and extensive histological changes which occur in stems of the bean (5, 4) and the tomato (1) when treated with lanolin mixtures of certain growth substances, and as a matter of general interest in studies on development, a study of the tissues of the pistil of *Ilex opaca* as affected by these compounds was undertaken.

The holly is dioecious and the pistillate plants, if isolated from possible pollination and otherwise untreated, do not set fruit. For the experiments here reported small pistillate plants with freshly opened flowers were divided into two lots, one of which was pollinated and the other sprayed once with a 0.04 percentage aqueous solution of indoleacetic acid. At frequent intervals from six to 571 hours after pollination or spraying, flowers or developing fruits together with their pedicels were taken from each lot and placed in Navashin's solution for subsequent histological preparation. Although the ap-

<sup>1</sup> Additional cost of publication sustained by the writers.

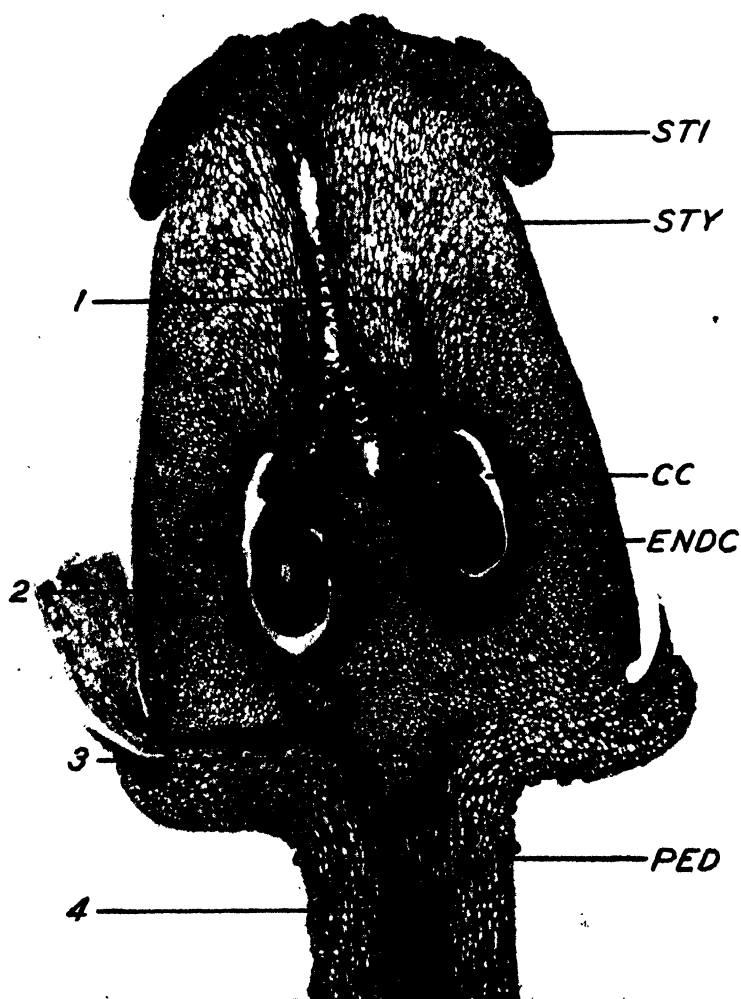


FIG. 1.—Young fruit of just opened flower previous to pollination (*st*, stigma; *sty*, style; *cc*, carpellary cavities; *endc*, endocarp of ovary; *ped*, pedicel). Numbers refer to levels at which sections were selected from the entire series through the fruits, referred to in following illustrations.

plication of other growth substances, namely indolepropionic, indolebutyric, and naphthaleneacetic acids, all resulted in parthenocarpic fruits, this histological study is confined to the effect of indoleacetic acid in comparison with pollination. The comparison likewise includes pistils of flowers which were neither sprayed nor pollinated, and which, in the normal course of events, would absciss after seven to ten days from full bloom without any apparent development of the ovaries. Such pistils were taken for study just prior to abscission.

The material was prepared according to the usual paraffin method, sectioned serially chiefly at  $10\ \mu$ , and stained in Flemming's triple stain.

The pistil of the holly consists of four undiverged carpels, each of which incloses a single ovule. The stigma is broad and slightly rounded, the style short. The vascular supply consists of four principal bundles each of which diverges to a sepal at the apex of the pedicel. Immediately above these and alternating with them are four additional bundles, each extending to a petal. Immediately above this level the vascular system is diverged into many small bundles arranged in four principal groups, each just below a carpellary cavity. These small bundles then extend upward and slightly outward through the inner portions of the carpellary walls, and end as groups of tracheids immediately below the stigma, which ranges from twenty to thirty cells in thickness in the just opened flower (figs. 1, 13).

If no pollen is applied to the stigma after the flower has opened there is practically no further development of any of the tissues composing the young fruit. After a week or ten days a yellowing and slight shriveling take place, the outer stigmatic cells collapse and darken, the ovules shrivel, and the entire fruit, including the pedicel, falls. Except for the slight shrinkage of the whole fruit which takes place, most of the cells which compose it seem still alive and relatively turgid; but the contents of the cells of the integument of the ovules are much plasmolyzed and the megagametophytes have partially disintegrated (figs. 4, 5, 13).

If pollen is applied to the stigma shortly after the petals expand, germination takes place promptly and the pollen tubes are clearly evident among the cells of the stigma and style by the end of 67



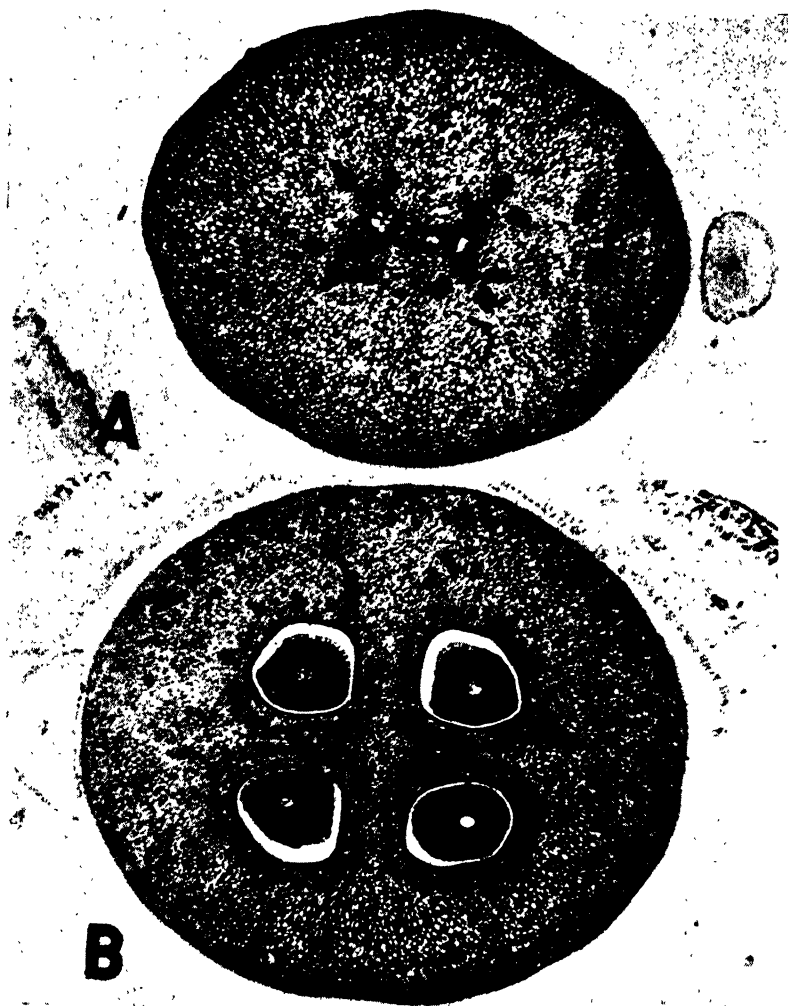


FIG. 2.—Just opened flower. *A*: section at level 1; *B*: at level 2. In *A*, vascular bundles just above each carpel and styler canal are shown. In *B*, beginning of differentiation of carpels into endocarp and exocarp and principal vascular bundles are evident, as well as transection of megagametophyte in each ovule.

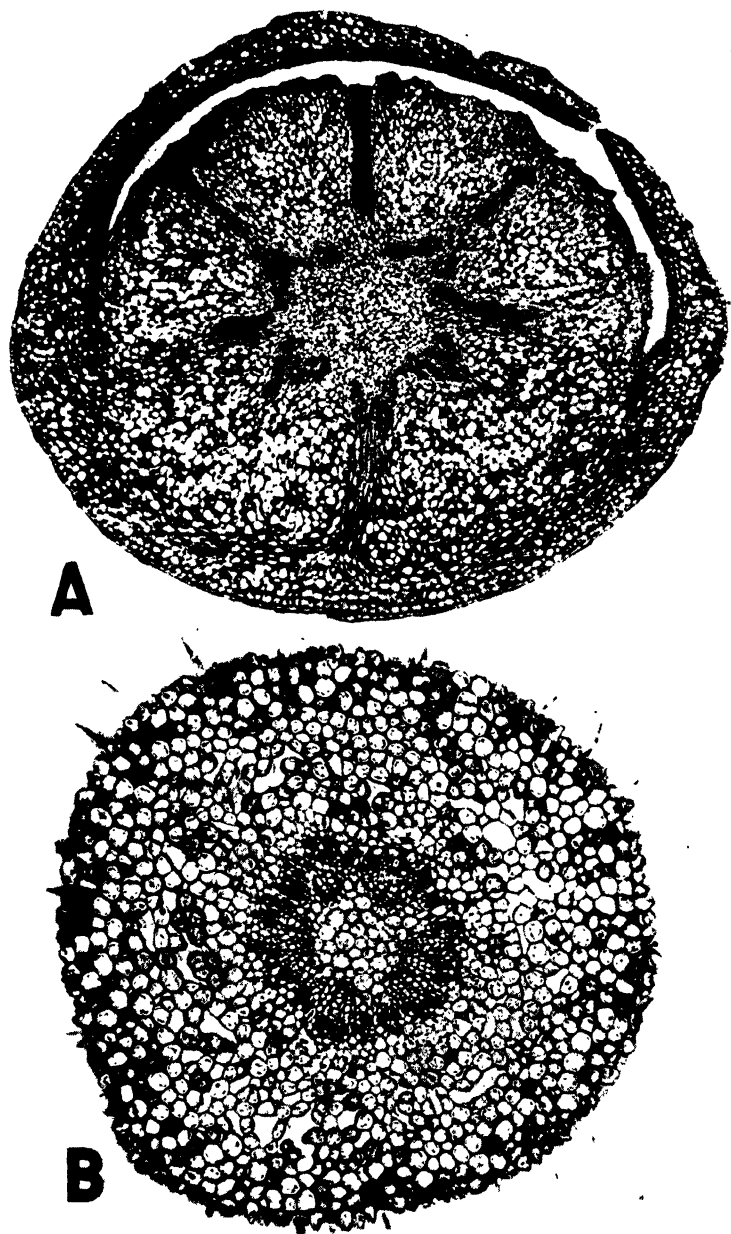


FIG. 3.—Just opened flower. *A*: at level 3; *B*: at level 4. In *A*, portions of vascular strands to petals and sepals as well as the four subcarpellary groups of vascular tissue are shown. *B* is considerably more enlarged than *A*.

hours. Fertilization occurs within 72 hours after pollination. When the pollen tubes begin to penetrate between the stigmatic cells the



FIG. 4.—Non-pollinated and non-sprayed fruit about to fall from the plant. Superficial cells of stigma are dead and ovules collapsed.

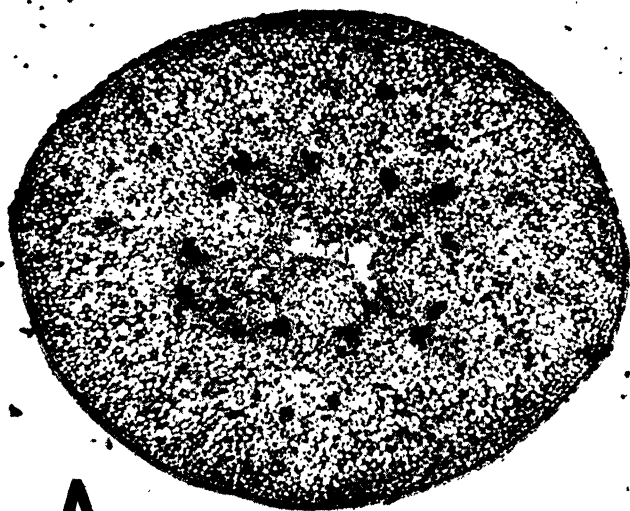
cells proliferate considerably. Those at the surface shrink and suberize somewhat, the others remain turgid and apparently unchanged for several days after fertilization.

Following fertilization the entire fruit becomes slightly greener;

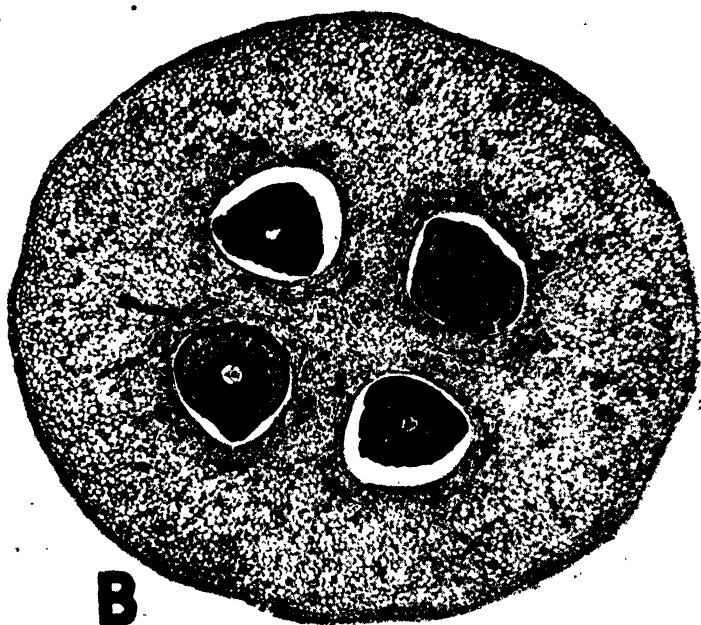
the parenchymatous cells of the style proliferate slightly but there is no marked activity of the cells of the vascular bundles. The cells of the ovaries enlarge considerably but do not divide extensively. There is no conspicuous proliferation of the cambium or other portion of the vascular bundles, the main changes being the addition of a few secondary xylem and phloem elements and maturation of the elements already differentiated. The cells of the style divide to a limited extent and enlarge appreciably; those of the ovaries multiply rapidly, especially those constituting the inner layers of the carpel walls adjacent to the carpellary cavities. By the end of 67 hours after pollination these latter cells have increased both in number and size, forcing the vascular bundles more widely apart. They constitute a well defined endocarp which by the end of 139 hours has started to become stony in contrast to the other cells of the carpels, which remain parenchymatous. By the end of 571 hours the endocarp has become so stony that it is difficult to section (figs. 10, 16).

Fertilization occurs by the end of 72 hours after pollination, and directly thereafter formation of the endosperm begins. Development proceeds rather slowly, however, so that by the end of 139 hours there are only a few cells present, although by the end of 571 hours the endosperm has become a conspicuous tissue composed of large thin-walled cells. The embryo is slow in developing and consists of very few cells at this same period. During development of the endosperm the cells of the integument divide somewhat extensively; the entire young seed elongates and enlarges; the epidermal cells of the integument become much enlarged and their walls become somewhat suberized. Material collected later than 571 hours after spraying has not yet been studied in detail.

The cells and elements composing the vascular bundles which extend mainly through the endocarp at its periphery enlarge and mature with the lapse of time. In the endocarpic portion of the fruit, cells of the vascular bundles increase more rapidly in number and mature more slowly than those which make up the bundles immediately below the carpels, those at the apex of the pedicel, or those in the pedicel itself. In fact there is but slight increase in the number of cells of the bundles below the carpels; as the fruit en-



**A**



**B**

FIG. 5.—*A*: section at level 1 and *B*: at level 2, of a fruit similar to one shown in fig. 4. Other than the collapsed megagametophytes and slightly shrunk ovules, tissues show very little change from those of the just opened flower.



FIG. 6.—*A*: section at level 1; *B*: at level 2, of fruit about to fall from the plant. No marked differences from the just opened flower could be detected.

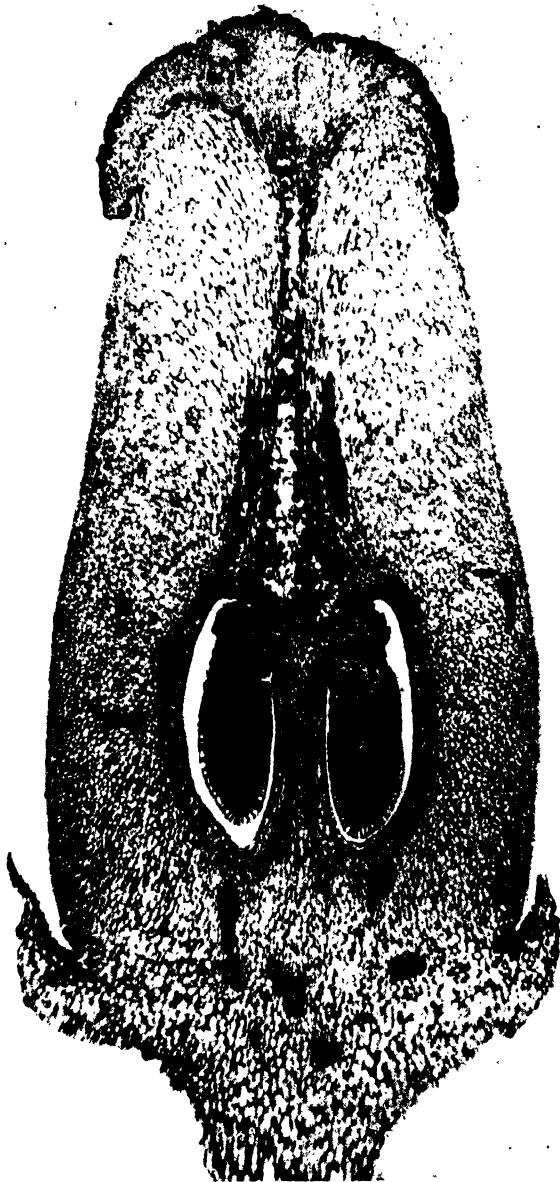


FIG. 7.—Young fruit 48 hours after spraying with indoleacetic acid in aqueous solution. Individual shown is a somewhat smaller specimen than the just opened flower in fig. 1. No significant differences from that stage are evident.



FIG. 8.—Young fruit 67 hours after spraying. Compared with just opened flower, there has been increased division of stigmatic cells, cells of the style, and to a lesser degree of the ovary.



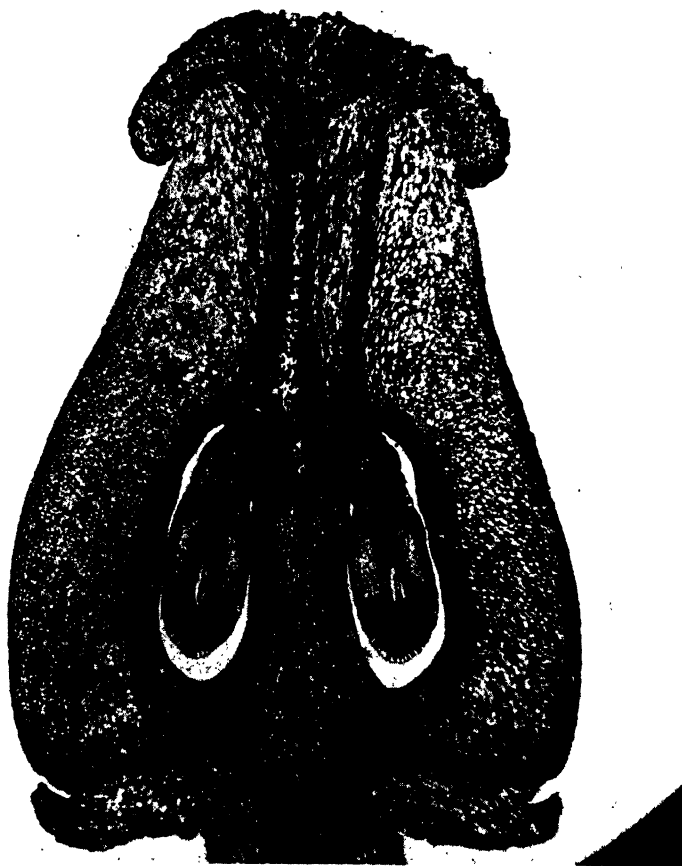


FIG. 9.—Young fruit 139 hours after pollination. Main changes which have taken place during period following pollination are a slight increase of the number and size of cells of the entire fruit, an increase in size of the vascular bundles, development of a few cells of the endosperm, and a considerable elongation and enlargement of the ovules.

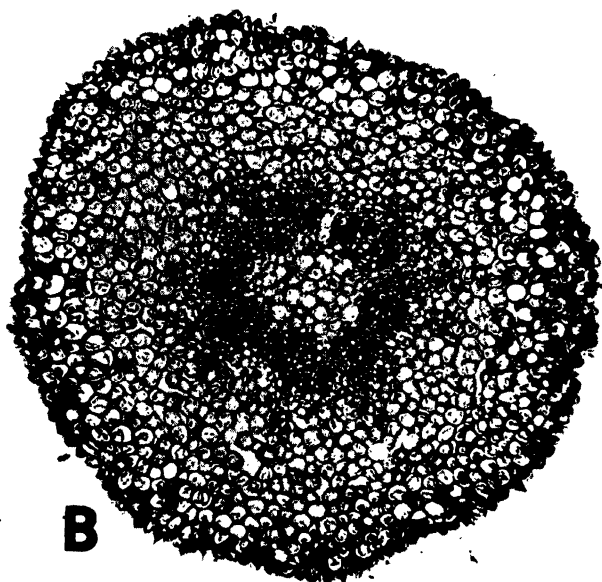
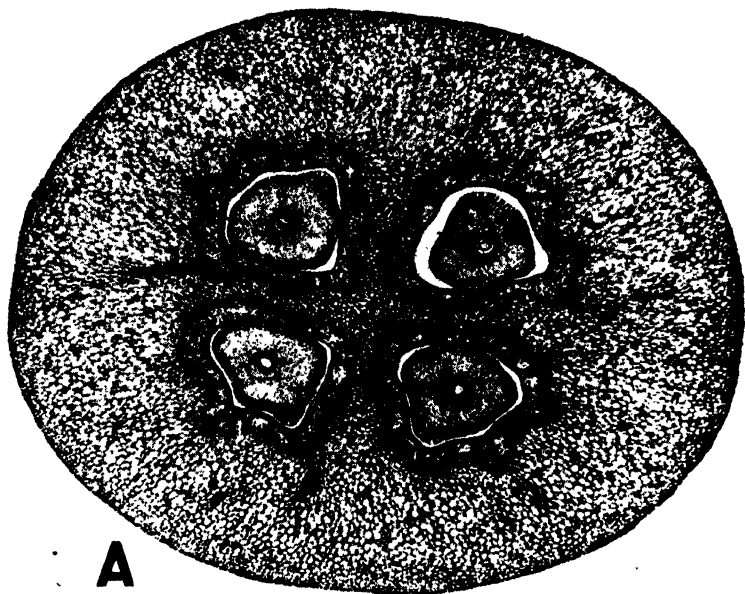


FIG. 10.—Fruit 139 hours after pollination. *A*: at level 1. Number of cells of endocarp has increased and their walls thickened appreciably. Cells of exocarp have enlarged and increased but slightly in numbers. Endosperm has begun development and cells of integument have increased in number and size. *B*: at level 4, more enlarged than *A*. Little or no change shown over just opened flower.

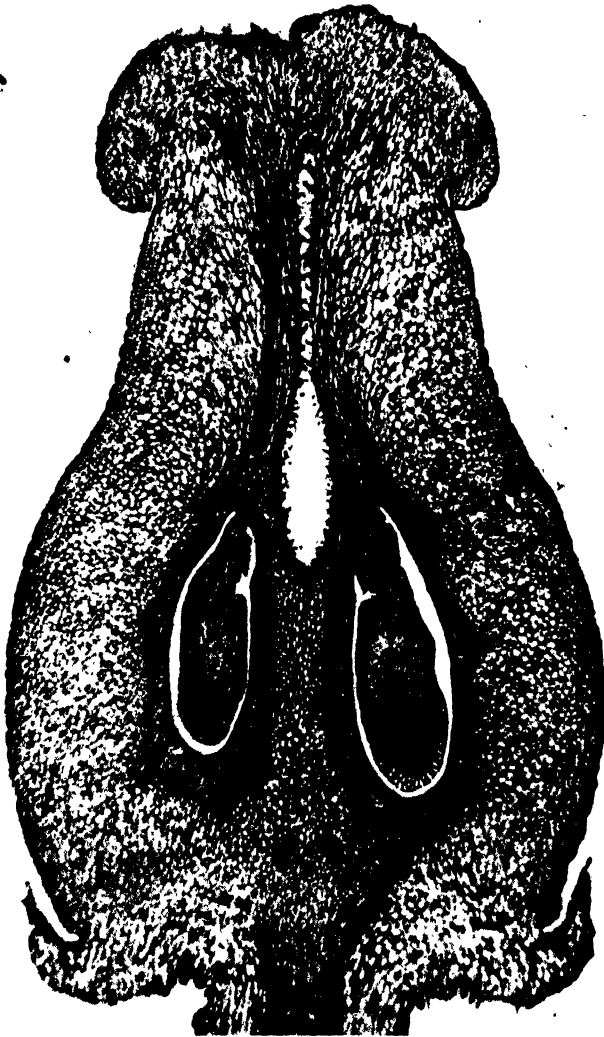


FIG. 11.—Fruit 158 hours after spraying. Similar in all respects to fruit 139 hours after pollination.

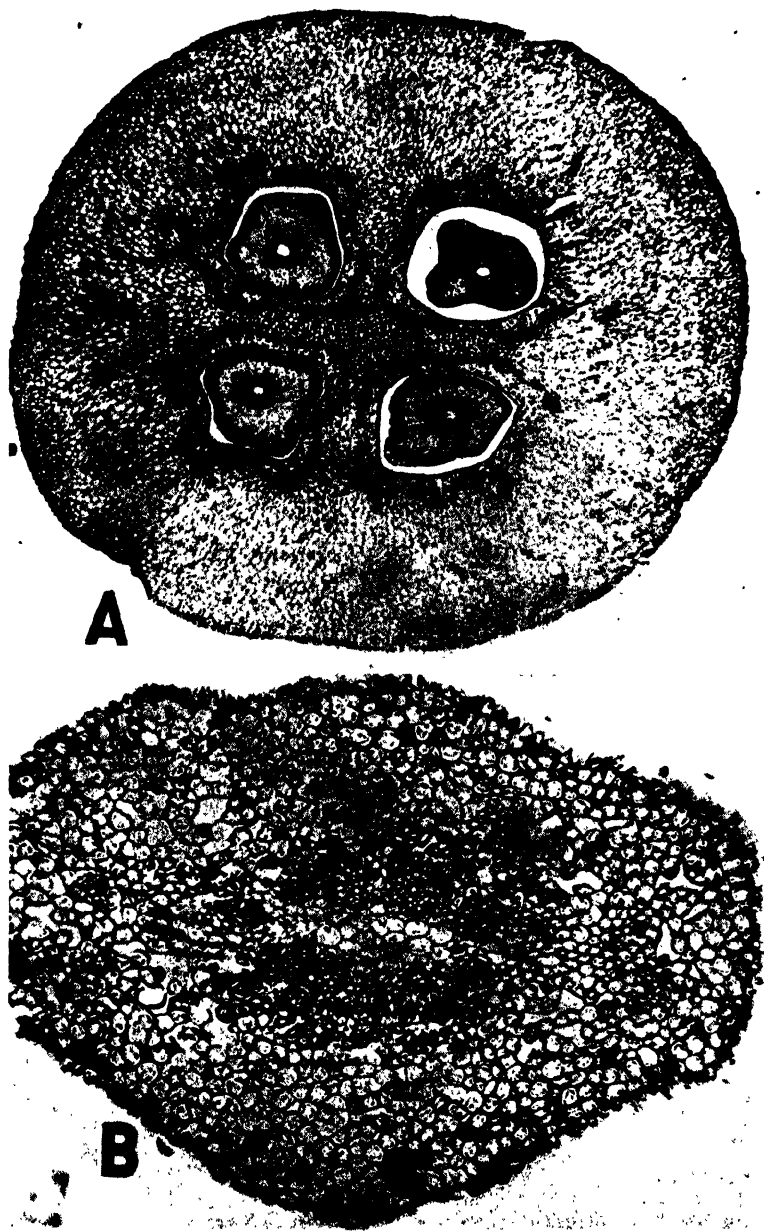


FIG. 12.—Fruit 158 hours after spraying. *A* shows similar changes to fig. 10*A* except that there is no endosperm present in the ovule and the gametophyte has disintegrated, leaving a cavity at center. *B*: similar to fig. 10*B*.



FIG. 13.—*A*: stigma and portion of style of just opened flower; *B*: same of fruit ready to fall. Superficial cells have collapsed, more or less suberized, and died; those below them show little change from the condition of those shown in *A*.

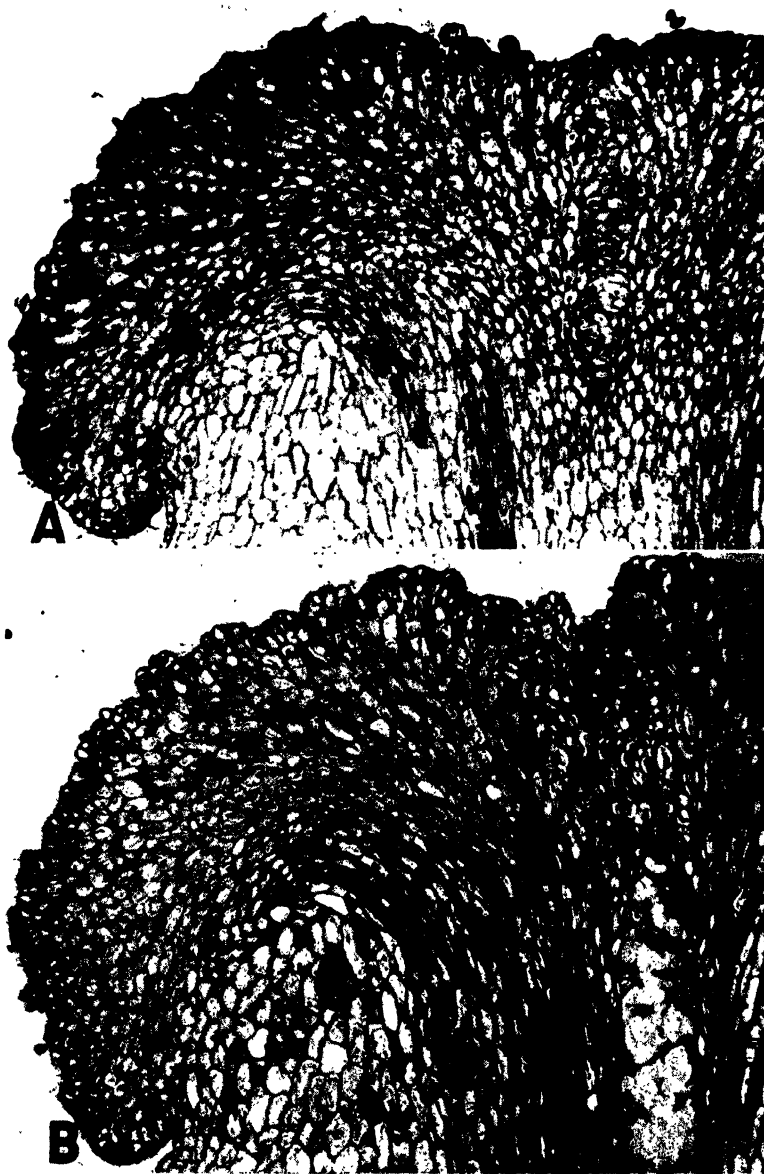


FIG. 14.—*A*: stigma 67 hours after pollination. Pollen grains evident at surface. Deeper stigmatic cells have dense contents, show a number of divisions, and no disintegration. The dark lines among them are pollen tubes. *B*: stigma 62 hours after spraying, similar in all respects to stigma shown at *A*, except that some cells have divided somewhat more extensively, are slightly larger, and their contents are slightly denser.

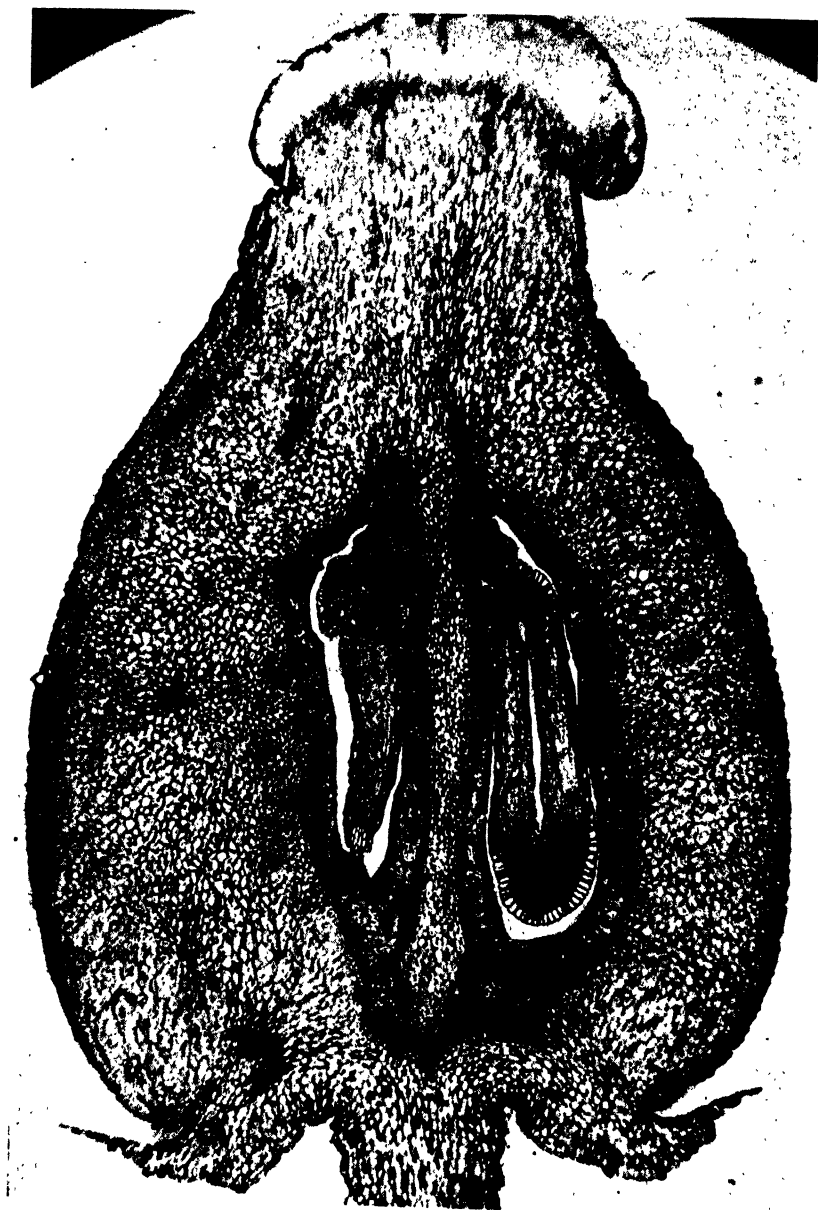


FIG. 15.—Fruit 302 hours after spraying. Developmental changes obvious, but continued development of ovule, containing neither gametophyte nor endosperm, is particularly apparent. At this stage the endocarp is slightly stony and strikingly delimited.



FIG. 16.—Fruit 571 hours after pollination. In carpel at right, the seed contains copious cellular endosperm at center, endocarp has become somewhat more stony, but is otherwise similar to figs. 15 and 17*A*.





FIG. 17.—A: 542 hours after spraying. At this time the fruits are nearly half the size they will attain at maturity. Cells of exocarp have enlarged but have multiplied little as compared with earlier stages; those of endocarp have multiplied more extensively and become somewhat stony but will become more so before maturity. Ovules are as large as seeds of pollinated fruits of same age, have a conspicuous cavity at center, and cells of integument are turgid. Ovule shows no tendency to collapse or disintegrate. The presence of five carpels is a somewhat common variation from the usual four. The tearing of the section occurred in cutting, owing mainly to the stony endocarp. B: 571 hours after pollination. These fruits are similar to those of comparable age developed following spraying; principal difference is presence of a copious endosperm in the seeds and greater firmness or thickness of walls of cells of the integument.

larges, however, the phloem and xylem elements, as well as the fibers, continue to mature. A few additional derivatives from the cambium are differentiated, but the changes which occur following pollination are largely those of maturation rather than increase in numbers.

Development of the parthenocarpic fruit following spraying with indoleacetic acid solution parallels almost precisely that following pollination. The chief differences are, first, the cells of the stigmas of the sprayed fruits proliferate somewhat more than those pollinated, and do not collapse and suberize quite so soon; and second, there is lack of development of an embryo or of endosperm in the ovules of the sprayed fruits. Particularly striking in the parthenocarpic fruits is the disintegration of the megagametophyte, which begins about five days after spraying despite the fact that the cells of the integument develop as rapidly and differentiate to the same degree as those of pollinated fruits, at least up to 302 hours. Disintegration of the megagametophyte leaves a large, long, central cavity extending nearly the entire length of the enlarging ovule, which as a whole shows practically no collapse at this time, nor even after 542 hours. This central cavity is much smaller than the one occupied by the developing endosperm of the seed of the pollinated fruit at an equivalent time period. The endocarp enlarges and hardens at about the same rate in the two types of fruits, and it is not possible to detect any direct outstanding differences in the vascular system, either in the fruit or in the pedicel (figs. 12, 15, 16, 17). The berries as a whole are as large, as turgid, and as green in one case as in the other. In the fall of the year no differences between the sprayed and pollinated lots were observed in time of fruit ripening, the green color of both having changed to red.

Particularly noteworthy is the lack of marked or disorderly cell proliferation in pistils to which indoleacetic acid had been applied. No unregulated cell division, tumorous outgrowths, nor root primordia, such as reported in stems of the bean and tomato under the influence of lanolin mixtures of indoleacetic acid, occur in the holly pistils when sprayed with aqueous solutions of this growth substance. Even when lanolin mixtures of much higher concentration are applied to the stigmas, parthenocarpic development closely

parallels that following pollination. Although the tissues of the young pistil might be expected to prove highly reactive because of their embryonic condition, they are not so in the sense that unusual structures are differentiated.

As in the case of fruits stimulated by pollination, the parthenocarpic fruits develop in a regular manner with swelling of the ovaries through increase in cell number and cell size, slight stimulation of the stigmatic cells followed by gradual shriveling later, and even normal development of the seed coat over a long period of time. No development of endosperm and no traces of embryo, however, are present within these seeds.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES  
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# VARIANT CHROMOSOME NUMBERS IN SPHAEROCARPOS

ELIZABETH L. MACKAY

(WITH TWENTY-FOUR FIGURES)

## Introduction

Since the first report by LORBEER (12) of the occurrence of diploid gametophytes of *Sphaerocarpos donnellii* Aust., a considerable number of heteroploid chromosome combinations have been shown to be consistent with the viability of gametophytes (typically having the chromosomal constitution  $A+X$  or  $A+Y$ <sup>1</sup>) and of sporophytes (typically  $2A+X+Y$ ).

The most frequent of these deviations from the ordinary chromosomal conditions follow upon the occasional formation of two instead of four spores from a single mother cell, as a result of some irregularity in the meiotic divisions. Usually both spores of a dyad so produced contain the sporophytic complement  $2A+X+Y$ . Their germination gives rise to diploid gametophytes female in appearance (12) but actually showing a measure of intersexuality (5, 6, 8). KNAPP (11) has found that in some families the two spores of a dyad may give rise either to two intersexual plants ( $2A+X+Y$ ) or to one male ( $2A+2Y$ ) and one female ( $2A+2X$ ).

As consequences of the formation of spores with more than the typical chromosome complement, of the union of gametes produced by heteroploid gametophytes, and of meiosis in heteroploid sporophytes, the following chromosome combinations have been shown to occur: Gametophytes: female,  $A+X$  (1, 2),  $2A+X$  (14),  $2A+2X$  (7); male,  $A+Y$  (1, 2),  $2A+2Y$  (8); intersexual,  $2A+X+Y$  (5, 6, 8, 12). Sporophytes:  $2A+X+Y$  (2, 12),  $3A+X+Y$  (14),  $3A+X+2Y$  (8, 14),  $3A+2X+Y$  (7, 14),  $4A+X+2Y$ ,  $4A+X+3Y$ . For the last two cited sporophytic complements, see a later page of the present paper.

<sup>1</sup> "A" throughout this paper refers to a single complete set of seven autosomes; "X" and "Y" indicate the respective sex chromosomes.

By the radiation of spore mother cells and of spores (10), and of the growing tips of female plants (13), gametophytes have been produced that were male although apparently having the female complement of A+X. In at least some such cases, KNAPP has found that a part of the X chromosome is lacking in the plant of "reversed" sex; and the same may be true in all cases in this category.

### Material and methods

Four classes of gametophytes of *Sphaerocarpos donnellii* were examined cytologically in the study here reported. Details regarding the origin of these plants are given in connection with the description of results obtained.

The fixatives used were Flemming's medium, Carnoy's alcohol-acetic, and Karpechenko's and Sax's modifications of Navashin's mixture. All gave satisfactory results, particularly if the air was removed from the material by suction when it was first placed in the fixative. Material was dehydrated in ethyl alcohol and imbedded in paraffin in the usual way. Staining was done with Delafield's haematoxylin, Heidenhain's iron-alum haematoxylin destained with picric acid or with 2 per cent iron alum, and SMITH's (15) modification of the crystal violet-iodine stain. Particularly satisfactory results were obtained by the last mentioned method.

Aceto-carminc smears were made of fresh material and of material fixed in modified Carnoy's solution. The latter gave the best of the few chromosome figures obtained by the smear method.

### Observations and discussion

#### MUTANTS

Thus far none of the clones of atypical form which have appeared in our cultures have been shown to possess other than the chromosome complement typical for the sex concerned. WOLFSON (16), for example, found that neither semi-sterile males, polycladous males, nor polycladous females display any visible chromosomal differences from typical males and females.

The cytology of two mutants not previously examined from this point of view is reported here. One is a dwarf ♂ clone (23.6002),

derived from a mating of typical ♀ 21.116 with the highly tufted ♂ 20.60 (4). This clone resembles a typical male in most respects, except that all the parts are smaller. In twenty matings with various female clones the dwarf male has proved sterile (9). It is found to have the haploid chromosome complement,  $7+Y$  (fig. 1); in no noticeable respect do its chromosomes differ from those of a typical male.

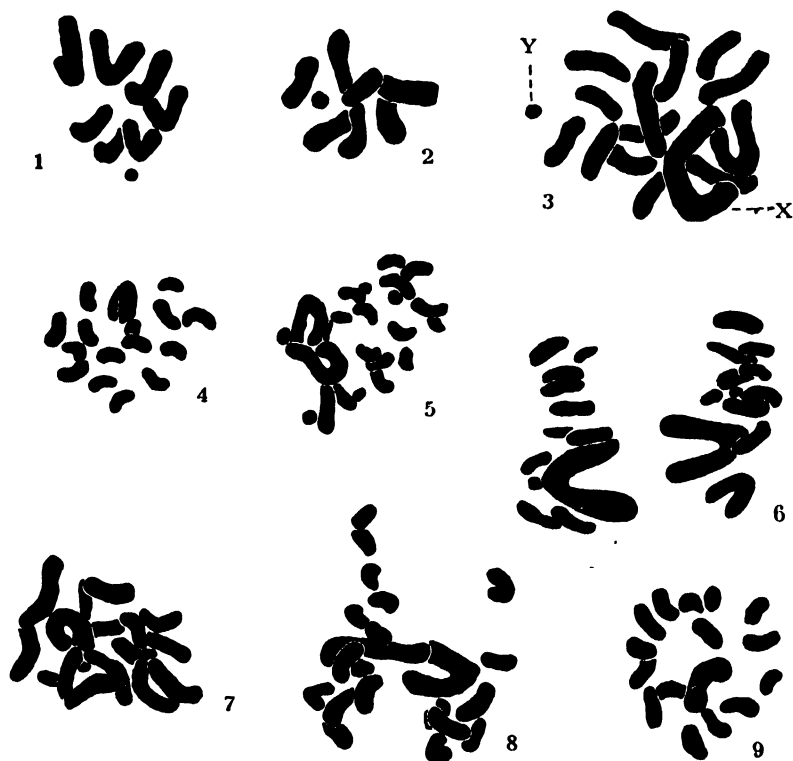
The other mutant studied (23.7094) came (3, 4) from a mating of the typical ♀ 20.205 with the polycladous (and genetically tufted) ♂ 20.254. The mutant clone bears approximately the same proportion of antheridia as a typical clone, but they are smaller and their involucre are in the main cup- or saucer-shaped instead of having the almost completely inclosing flask-shape of typical males. Most of the antheridia are hence partly or entirely exposed. The character of the involucre suggested the designation cupulate for this mutant. It has proved to be fertile, producing sporophytes in seven of thirty-nine matings with various females (9); but none of the gametophytic progeny have shown the cupulate character. This cupulate mutant also has the haploid chromosome complement of a typical male,  $7+Y$  (fig. 2).

#### GAMETOPHYTES FROM SPORES OF DYADS

In general, as already noted, the spores of the dyads which occasionally replace tetrads in the adherent-spored races of *S. donnellii* are diploid, giving rise to intersexual gametophytes with a chromosome complement of  $2A+X+Y$ . A study of the gametophytic clones developing from such spores has given the following results. The clones referred to later as female are those apparently such, without reference to their possibly intersexual character.

Clone 35.2201.—This female clone developed from a spore of a dyad of a sporophyte, the offspring of a mating of a typical ♀ (31.1019) with the cupulate ♂ (23.7094) previously mentioned. In a previous paper (14) clone 35.2201 was tentatively reported as having  $2A+X$  chromosomes; later study has revealed the presence of a  $Y$ . In the prophase group shown in figure 3 the  $X$  is evident, and the  $Y$  is much smaller than any of the autosomes. In figure 4, showing an anaphase group from the same clone, a  $Y$  cannot be

certainly distinguished, although there are sixteen chromosomes present including the X; in the anaphases the X seems often to approach more nearly the size of the autosomes than at other



FIGS. 1-9.—*Sphaerocarpus donnellii*: Fig. 1, dwarf ♂ clone 23.6002, anaphase group showing eight chromosomes (A+Y). Fig. 2, cupulate ♂ clone 23.7094, prophase (A+Y). Figs. 3-6, ♀ clone 35.2201 (2A+X+Y): fig. 3, prophase, X and Y very evident; fig. 4, anaphase group, Y not clearly distinguishable; fig. 5, meta-anaphase in lateral view with lagging X, not all autosomes visible; fig. 6, lateral view of anaphase, some autosomes not in section. Figs. 7, 8, ♀ clone 36.1 (2A+X+Y): fig. 7, prophase; fig. 8, lateral view of anaphase showing part of autosomes and lagging X. Fig. 9, ♀ clone 37.3, diploid with one X. (Figs. 1, 2 from androgonia; figs. 3, 5, 7, 9 from involucre cells; figs. 4, 8 from young archegonium; fig. 6 from thallus cell.) All Xc. 3600.

stages of division. In figures 5 and 6, showing lateral views of anaphases in the same clone, the single X shows its characteristic lagging behavior; not all the autosomes are visible. The eggs produced by this clone are fertile, as shown by several matings which

have produced sporophytes; one was with the diploid ♂ 32.77 ( $2A+2Y$ ) from which were produced (presumably) tetraploid sporophytes ( $4A+X+3Y$ ).

It has been noted (8) that certain races, at least under greenhouse conditions, produce unusual proportions of spore dyads, and that there is evidence of the inheritance of the dyad-producing tendency. The clones referred to later under the present heading were representatives of such a dyad-producing race.

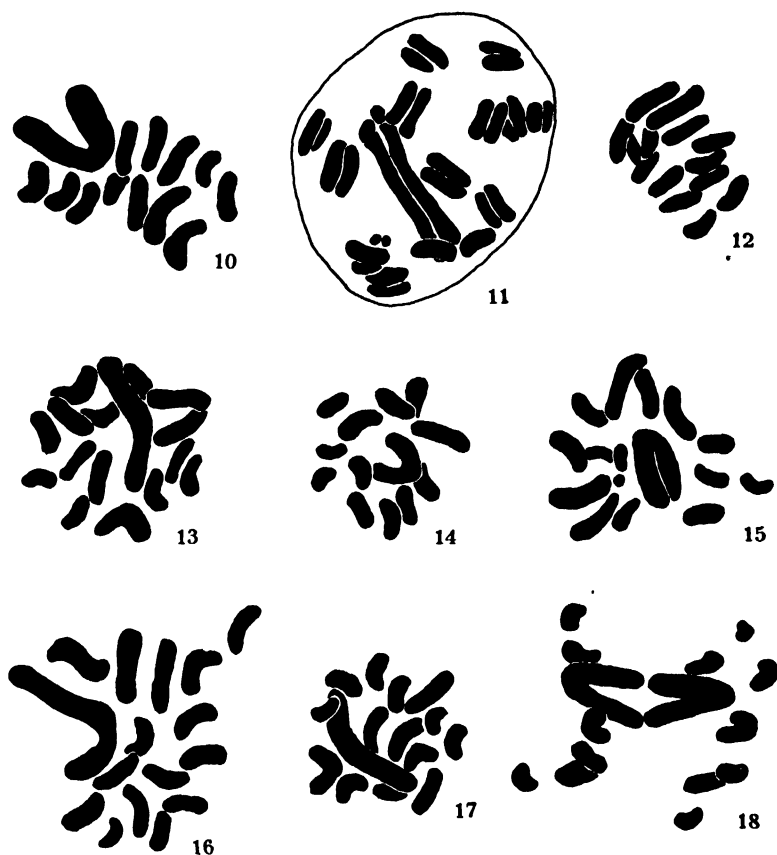
Clone 36.1, from the mating  $31.417 \times 31.789$ .—This clone is female, with  $2A+X+Y$  chromosomes (fig. 7). The lagging of the X is evident in figure 8.

Female clones 37.3, 37.5, 37.9, and 37.201, all from the mating  $31.742 \times 31.706$ .—Clones 37.3 (fig. 9) and 37.5 (fig. 10) are at least approximately diploid; an X is present in each clone, and probably one of the sixteen chromosomes of clone 37.3 shown in figure 9 is the Y; not all the chromosomes are present in figure 10 (from clone 37.5). An unusual prophase figure (fig. 11) was observed in the nucleus of the central cell of an archegonium in clone 37.9; this shows very distinctly fourteen double chromosomes (or twenty-eight paired chromosomes) including an X and a Y; the double nature of the other two chromosomes does not show in the plane of the section. No figure similar to this has appeared in any other mitosis. The appearance cannot be explained by the fixative or stain used, which were the same as have been used consistently throughout the study. Clone 37.201 is from a mating the sporophytes in which were irradiated with soft X (Grenz) rays shortly after the conclusion of meiosis. In the material available of this clone chromosome figures were few; the most satisfactory one found (fig. 12) indicates that the clone is approximately diploid, but gives no information concerning the presence of an X or of a Y chromosome.

Female clones 37.803, 37.805, 37.807, 37.809, from the mating  $31.427 \times 31.789$ .—Clone 37.803 has probably a  $2A+X+Y$  chromosome complement (fig. 13). The approximate diploid number appears in clone 37.805 (fig. 14). Clone 37.807 has  $2A+X+Y$  chromosomes (fig. 15). In an aceto-carmin preparation (fig. 16) the chromosome complement of 37.809 was found to be sixteen (diploid) with one X and probably one Y; but since several of the smaller



chromosomes were all so nearly of one size the Y is not certainly recognizable.

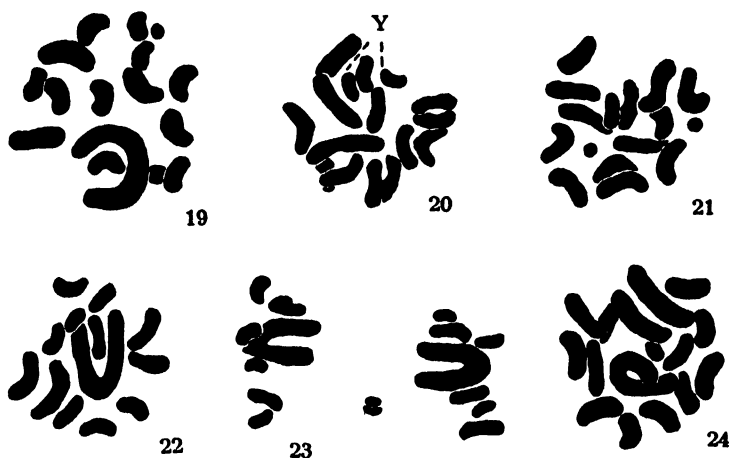


FIGS. 10-18. —*Sphaerocarpos donnellii*, ♀: Fig. 10, clone 37.5, prophase group, approximately diploid with one X. Fig. 11, prophase in clone 37.9, chromosomes clearly double or paired ( $2A+X+Y$ ). Fig. 12, clone 37.201, prophase showing approximate diploid complement. Fig. 13, prophase from clone 37.803 ( $2A+X+Y$ ). Fig. 14, clone 37.805, anaphase with one X. Fig. 15, clone 37.807, prophase group ( $2A+X+Y$ ). Fig. 16, prophase from clone 37.809, aceto-carmin preparation ( $2A+X+Y$ ). Fig. 17, clone 37.3207, prophase with one X. Fig. 18, clone 37.3214, anaphase, probably diploid, with lagging X. (Fig. 11 from central cell of archegonium; figs. 13, 14, 17 from involucre cells; others from thallus cells.) All  $\times c. 3600$ .

Clones 37.3207 and 37.3214, from the mating  $31.427 \times 31.689$ .—These female gametophytes have approximately the diploid number

with one X (figs. 17, 18). Since the very small Y is so easily obscured from view by an autosome or particularly by the X, it cannot definitely be concluded from the few available figures of these clones that a Y is absent.

All the female or apparently female gametophytes produced from dyad spores were thus found to have the diploid or approximately the diploid number of chromosomes, including one X, and in most cases a Y also was shown to be present. This is in agreement with the observations of LORBEER (12) and ALLEN (5, 6, 8).



FIGS. 19-24.—*Sphaerocarpos donnellii*: Fig. 19, ♀ clone 36.201, prophase with  $2A+X+Y$  chromosomes. Fig. 20, ♂ clone 37.4801, polar view of equatorial plate ( $2A+2Y$ ). Fig. 21, ♂ clone 37.4859, polar view of equatorial plate ( $2A+2Y$ ). Fig. 22, ♀ clone 37.4821, prophase from aceto-carmin preparation showing approximate diploid chromosome number with one X. Fig. 23, metaphase from clone 37.4822, approximately diploid with one X and possibly one Y pair (autosomes not all in plane of section). Fig. 24, ♀ clone 37.4925, prophase ( $2A+X+Y$ ). (Figs. 20, 21 from androgonia; fig. 24 from involucre cell; others from thallus cells.) All  $\times c. 3600$ .

#### OFFSPRING OF A TRIPLOID SPOROPHYTE

Clone 36.201.—This is a female clone derived from a tetrad spore borne by the triploid sporophyte resulting from a mating of clone 30.1004 ( $2A+2X$ ) with clone 33.53 ( $A+Y$ ). This clone has  $2A+X+Y$  chromosomes (fig. 19). This agrees with previous results (14) showing that spores strictly diploid in terms of the autosomes may be borne by triploid sporophytes, and suggesting that

hypohaploid and hyperhaploid, hypodiploid and hyperdiploid auto-some combinations are non-viable or but feebly viable. Hyperhaploid chromosome complements have been found by KNAPP (11) to be non-viable or unstable.

#### OFFSPRING OF A TETRAPLOID SPOROPHYTE

Numerous viable spores (in tetrads) were produced by sporophytes resulting from a mating of diploid ♀ 32.65 ( $2A+X$ ) with diploid ♂ 32.77 ( $2A+2Y$ ). The sporophytes were presumably tetraploid with a complement of  $4A+X+2Y$ . Of the gametophytic progeny, the complements of two ♂ clones (37.4801, 37.4859) and three ♀ clones (37.4821, 37.4822, 37.4925) have been determined. Each of the males is diploid with two Y chromosomes (figs. 20, 21). They make a total thus far known of four *S. donnellii* males with  $2A+2Y$  chromosomes. Two of the three females (clones 37.4821, 37.4822) are at least approximately diploid with one X (figs. 22, 23). It may be conjectured that the lagging chromosomes seen in the anaphase shown in figure 23 are Y's, but not enough figures have been seen to verify this assumption. A more definite count was obtained in female clone 37.4925 of the same family (fig. 24); this has  $2A+X+Y$  chromosomes. One intersexual organ was observed in clone 37.4821. So far as these results go, it appears that meiosis in tetraploid sporophytes is regular or approximately so, diploid spores being produced.

Tetraploid sporophytes from the mating 35.2201  $\times$  32.77, mentioned earlier, with presumably  $4A+X+3Y$  chromosomes, had few progeny and those surviving are only feebly viable. Their feebleness may be due to irregularities in meiosis resulting in the production of less viable aneuploid forms.

#### Summary.

1. A dwarf and a cupulate male mutant, which developed from spores of pedigreed sporophytes, have in each case a haploid chromosome complement of seven autosomes and one Y chromosome.

2. In harmony with previous results, gametophytes developed from spores of dyads are diploid, or approximately diploid, with (in the cases studied) one X and (usually at least) one Y chromosome.

3. The viable progeny of a triploid sporophyte possess, so far as has been found, no irregular chromosome complements; in each of the clones of such origin studied the diploid autosome number (14) was present.

4. In the cases studied, gametophytic offspring of tetraploid sporophytes are diploid; the males have two Y chromosomes; the apparent females have  $2A+X+Y$ ; the latter are probably (in one case certainly) intersexual.

5. Additional viable sporophytic chromosome complements here reported are  $4A+X+2Y$  and  $4A+X+3Y$ , those previously observed being respectively  $2A+X+Y$ ,  $3A+X+Y$ ,  $3A+X+2Y$ , and  $3A+2X+Y$ .

The writer expresses her appreciation to Professor C. E. ALLEN, who suggested the subject of this research and gave freely of his time and criticism during its progress and in the writing of this paper. The work has been done under a grant from the Wisconsin Alumni Research Foundation.

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CYTOLOGICAL STUDIES IN THE MYRICACEAE  
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 485

JAMES STOKES

(WITH FORTY FIGURES)

Introduction

According to SMALL (4) the genera *Myrica* and *Cerothamnus* are dioecious and *Comptonia* is monoecious. Partly for this reason the Myricaceae were selected for cytological study in order particularly to determine whether heterochromosomes are present. Inasmuch as such elements were not found, the meiotic processes in the microspore mother cells were studied in the following species: *Myrica cerifera* L., *M. pumila* Michx., *M. carolinensis* Mill., and *Comptonia peregrina* (L.) Coulter.

Plants of *M. cerifera*, *M. pumila*, and *M. carolinensis* were collected in the vicinity of Valdosta, Georgia. *M. pumila* is usually found on sandy acid pinelands. The distribution of *M. cerifera* overlaps that of *M. pumila*; it is common also in hammocks and swamps. *M. carolinensis* is restricted to low or wet acid pinelands, edges of hammocks, and stream banks.

Field observations on these species indicated that all are strictly dioecious. In order to check the field observations more carefully, representative specimens of *M. cerifera*, which were sufficiently mature to have produced strobili the previous season, were transplanted to the campus of the Georgia State Womans College. Although the new environment was somewhat drier than that of their native habitat, they grew luxuriantly.

Microscopic examination of staminate strobili of various ages gave no evidence of the presence of carpellate flowers. Although a majority of the carpellate strobili of a particular plant proved to be strictly carpellate, others were androgynous. In the latter the staminate flowers developed among the carpellate long after the disappearance of the staminate strobili of the strictly staminate plants. The anthers of such flowers were well developed and contained ap-

parently functional pollen. All variations observed were in the direction of carpellate to staminate. No perfect flowers were observed among either staminate or carpellate strobili. In all three species staminate strobili developed much earlier than the carpellate.

Specimens of *Comptonia peregrina* were located in the vicinity of Thornton, Illinois. Macroscopic examination indicated that the plants were monoecious. Microscopic examination of staminate strobili showed no carpellate flowers, but many of the carpellate strobili were androgynous. In such androgynous strobili staminate flowers appear among the carpellate during the late stages of seed development. In *Comptonia*, as in *Myrica*, the staminate strobili develop earlier than the carpellate.

Previous investigations of various species of *Myrica* have shown that variations in sexual expression are not infrequent. DAVEY and GIBSON (3) found that in *M. gale* there always exists a small proportion of monoecious plants exhibiting all gradations between strictly staminate and strictly pistillate. The series includes plants or shoots (a) bearing staminate and pistillate catkins of the normal type, (b) bearing androgynous catkins, (c) the bulk of the catkins consisting of hermaphroditic flowers. These writers state (3): "It has been found that the sex (if it may be so termed) of a bush or shoot may vary from year to year. The variations observed during several years have been almost entirely in the direction of change from the pistillate to the staminate condition; but in the present season (1916) several instances of the reverse change have been noted."

CHEVALIER (1) examined certain monoecious species of this family, notably *Myrica californica*, *M. conifera*, and *M. pubescens*. He reported variations in these forms similar to those reported by DAVEY and GIBSON for *M. gale*. The causal complex underlying variations in sexual expression has not been determined. CHEVALIER suggested nutrition as the factor controlling the expressing of sex in the monoecious forms examined. In view of the results cited, as well as of my own observations, strict dioecism is not to be expected among the Myricaceae.

The Myricaceae might afford excellent material for studies in the relation of environment to variations in sexual expression.

### Material and methods

Staminate strobili were collected in the field. Before fixation they were cut into small pieces, and in some cases individual flowers were fixed. Flemming's strong, medium, and weaker solutions were used. The medium solution gave satisfactory results. In addition, a variety of other fixatives such as Carnoy's, La Cour's, and various modifications of Navashin's solution were utilized. Satisfactory fixations were obtained with Sax's modification of Navashin's solution.

Belling's iron-aceto-carmine method proved effective in the choice of materials to be fixed for studies of chromosome numbers, but proved ineffective as a quick method of obtaining counts, as the chromosomes failed to stain sufficiently to render them clearly visible. The most satisfactory staining results were obtained with Heidenhain's iron-alum haematoxylin stain.

### Investigation

#### *Myrica cerifera*

**EARLY PROPHASE.**—The cells of the primary sporogenous tissue undergo division in all planes, ultimately giving rise to a cylindrical mass. After completion of these premeiotic divisions, the definitive pollen mother cells enter upon their long period of growth.

The resting microspore mother cells are polyhedral and are readily distinguishable from adjacent cells by the size of their nuclei, the affinity of the nucleoli for organic dyes, and the density of their cytoplasm. The cell wall is thin; the cytoplasm is rather dense and granular. The nucleus is almost circular in cross section. The nucleolus stains sharply, in contrast with the remaining apparently rather scanty chromatic material. The chromatic threads appear irregular in outline and exhibit thickenings where they intersect (fig. 1).

The first indication of the beginning of the leptotene is a gradual thickening of some of the chromatic fibers. The netlike structure of the resting nucleus is replaced by long, thin, delicate threads so intertwined that individual ones cannot be followed throughout their total length (fig. 2). The leptotene stage is of short duration and the chromatic threads are too delicate to allow detailed observations



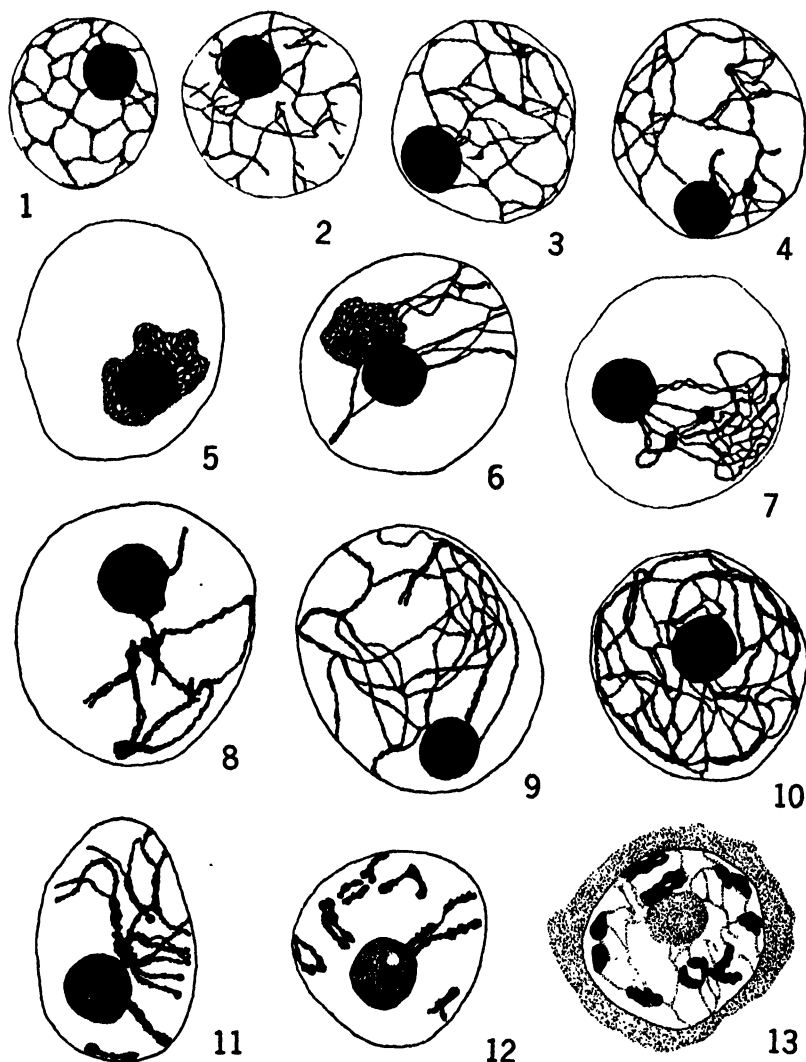
of their behavior. There is scanty evidence of pairing (fig. 3), but figure 4 may be interpreted as the initiation of the zygotene stage.

**ZYGOTENE TO DIAKINESIS.**—Soon the chromatic threads withdraw from the nuclear membrane and the nucleus passes into the condition of synizesis (fig. 5). Synizesis, whether an artifact or not, is of long duration. Extra nucleoli are not present at this or at subsequent stages of the first meiotic division. In most cases observed the synizetic knot is composed of an irregular mass of threads enveloping the nucleolus. All observations indicate that the nucleolus is never entirely free of the threads; at least one pair remains closely associated with this body, and possibly attached to it.

Evidence of the paired condition is more definite during the loosening of the synizetic knot (figs. 6, 7). The extension of loops or twists of the threads into the nuclear cavity is the first indication of the loosening of the knot, and apparently it occurs slowly. During early pachytene the bivalent threads lie irregularly distributed in the nucleus (figs. 8–10). They now become somewhat thicker, but no free ends are observable other than those which are due to cutting.

The transition from pachytene to diplotene is not readily observed in this material. The long threads characteristic of early pachytene undergo further shortening and thickening. The paired threads, which diverge widely at certain points, are held together by chiasmata (fig. 11). The doubleness of each chromosome of the bivalents, commonly observable in plant material with long chromosomes, was not seen in this material, which has very short chromosomes. The bivalent chromosomes now become progressively shorter and thicker and there is evident clumping and condensation of the chromatic materials as diakinesis is reached (fig. 12).

**DIAKINESIS.**—At diakinesis there is still no indication of the tetrad condition of the bivalents. The extreme shortening and thickening completely obscure the chromatids. In earliest stages of diakinesis the bivalents are still somewhat extended (fig. 12), but gradually the chromosomes of each pair approach each other very closely (fig. 13). The components of some bivalents are joined end to end, while others appear as V's and X's. At diakinesis the eight bivalents are peripherally placed in the nucleus. While this material, with very small and short chromosomes, is unsuitable for



FIGS. 1-13.\*—*Myrica cerifera*: fig. 1, premeiotic nucleus of pollen mother cell; fig. 2, leptotene threads; fig. 3, early zygotene; fig. 4, pairing of threads in zygotene; fig. 5, synizetis; fig. 6, opening of synizetic knot; fig. 7, early pachytene; figs. 8-10, pachytene stages; fig. 11, diplotene stage with chiasmata; fig. 12, early diakinesis; fig. 13, late diakinesis showing eight bivalents.

\* All figures drawn with 2 mm. apochromatic objective and 30X compensating ocular, at table level with the aid of a camera lucida.

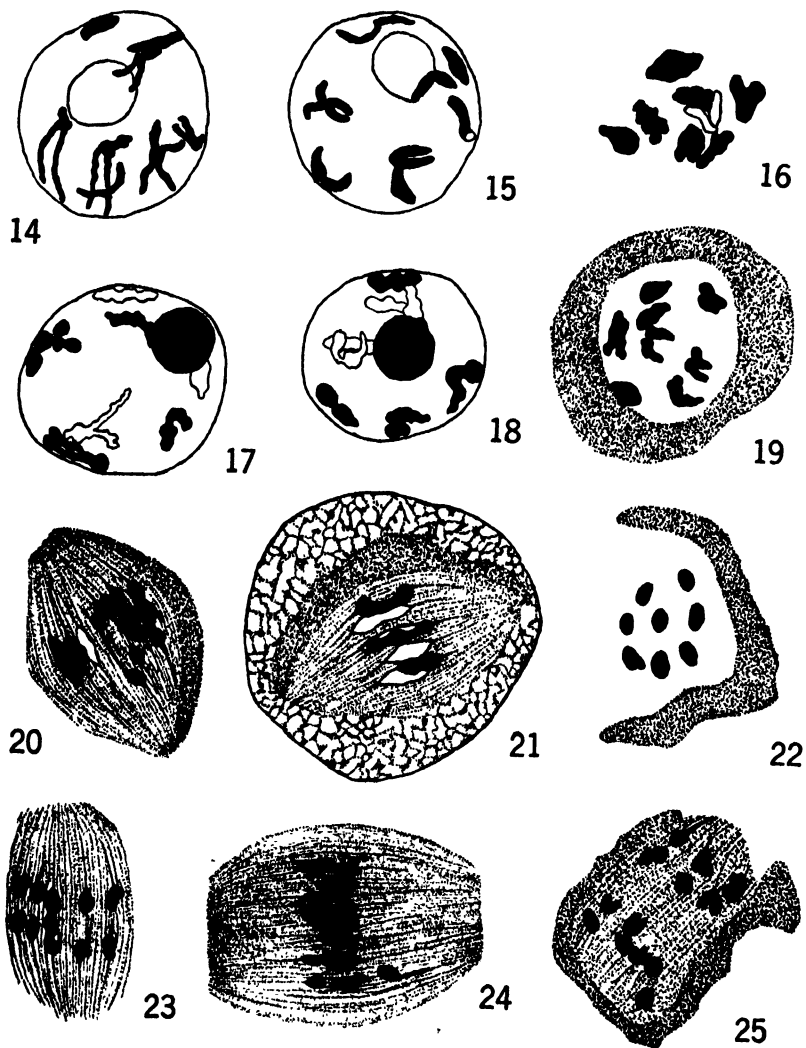
a study of chiasma formation and behavior, figures 11-13 give evidence of their existence.

During the diakinetik stages the cytoplasm of the microspore mother cell has been undergoing changes. The peripheral region of the cytoplasm has become more vacuolate and a zone of granules of various sizes in the inner portion forms a definite perinuclear zone. The outer boundary of this zone is somewhat irregular in outline; its inner boundary adjoins the nuclear membrane (fig. 13).

The nucleolus, which during diakinesis has retained its affinity for organic dyes and has occupied a position at one side of the nucleus, gradually loses its staining capacity and begins to disappear. Nucleolar budding does not accompany the disorganization process. Shortly after the disappearance of the nucleolus the nuclear membrane becomes irregular in outline and begins to disintegrate. The nuclear cavity decreases in size, and the condensed bivalents pass from the peripheral region of the nucleus toward its center.

Spindle fibers appear in the nuclear cavity about and among the chromosomes. Some of these fibers appear to become attached to the bivalents at or near their ends (fig. 21) while others extend between the poles and remain unattached to the chromosomes. A typical multipolar diarch spindle is formed, and the bivalents, now closely grouped together, lie at the equator. In some cases the spindle appears pointed at the poles while in others it is rather broad.

**FIRST METAPHASE TO END OF INTERKINESIS.**—The bivalents are now arranged upon the equatorial plate (fig. 21). At this stage the chiasmata have become nearly or completely terminalized (figs. 20, 21). During the metaphase of the first meiotic division the chromosomes show no evidence of being composed of two chromatids (fig. 21). Gradually the homologous members of each bivalent move away from each other, the movement beginning at the spindle attachment regions. As a consequence of terminal spindle attachment and the probable resistance of chiasmata to the forces of disjunction, some of the chromosomes are drawn out into fine threads (fig. 20). The bivalents do not disjoin simultaneously. During the anaphase the two chromatids of each chromosome become visible (fig. 25) and in many of the dyads the two chromatids remain in contact



FIGS. 14-25.—Figs. 14-16, *Myrica carolinensis*: 14, 15, diakinetic stages; 16, late diakinesis showing eight bivalents. Figs. 17-19, *M. pumila*: 17, 18, diakinetic stages; 19, late diakinesis showing eight bivalents. Figs. 20-23, *M. cerifera*: 20, precocious disjunction of two bivalents at first metaphase; 21, cell with spindle figure showing relative size of spindle and cell; chromosomes in metaphase; 22, polar view of metaphase plate with eight bivalents; 23, early first anaphase. Fig. 24, *Comptonia peregrina*: spindle with chromosomes in early first anaphase. Fig. 25, *M. cerifera*: late first anaphase showing dyads.

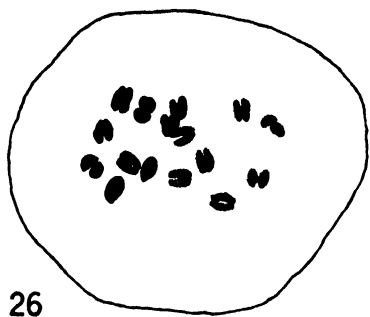
at the terminal attachment and diverge at the free ends, thus resulting in V-shaped figures (fig. 26). Upon reaching the poles the chromosomes usually mass together so closely that their individual identities cannot be distinguished. The aggregate of chromosomes later begins a loosening process and globular chromatic masses appear. Some of these bodies are nucleoli (fig. 27).

A nuclear membrane forms about each daughter nucleus and one or more nucleoli appear. During interkinesis the chromatic masses in the daughter nuclei continue the loosening process until the globular bodies disappear and the chromatic material is distributed on anastomosing threads. The spindle disappears with no indication of cell plate formation. The remains of the perinuclear zone are present between the daughter nuclei and contain numerous granules (fig. 27). At the termination of interkinesis small chromosomes appear in each daughter nucleus (fig. 28).

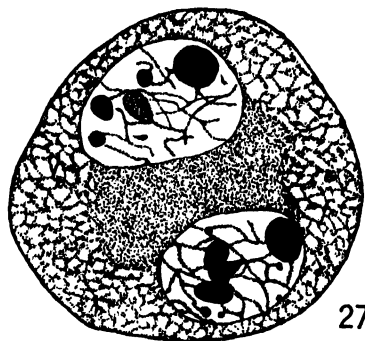
SUGIURA (5) reported the occurrence in *M. rubra* of an occasional constriction of the mother cell during interkinesis, but no incipient furrowing of the protoplast at interkinesis was observed in any of the species reported in this paper.

SECOND MEIOTIC DIVISION.—The spindles of the second meiotic division appear in the region previously occupied by the daughter nuclei, and are similar in appearance to those of the first division. The two spindles may lie parallel to each other (fig. 34) but more commonly they are at right or oblique angles. The surrounding cytoplasm contains many granules. As the chromosomes pass to the equatorial plates their double nature is evident (figs. 35, 36). Polar views of the four anaphase plates establish the haploid number as eight (fig. 37). After reaching the poles the chromosomes approach one another and form dense chromatic masses. About each polar group a nuclear membrane is formed (fig. 39). As the nuclei grow, the chromatic masses become loosened and anastomosing threads form. A nucleolus reappears in each nucleus. Continued loosening results in a resting nuclear condition characterized by a netlike arrangement of the threads (fig. 40).

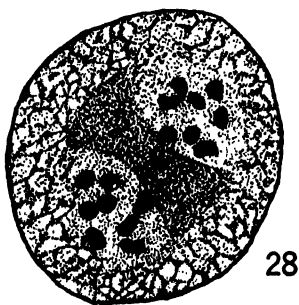
The second division spindles persist until the formation of the nuclear membranes of the daughter nuclei and the region between the spindles is occupied with granules of variable sizes. Cell plates



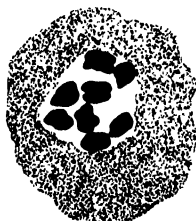
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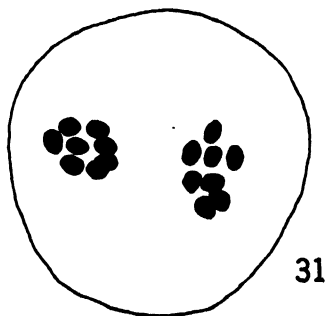
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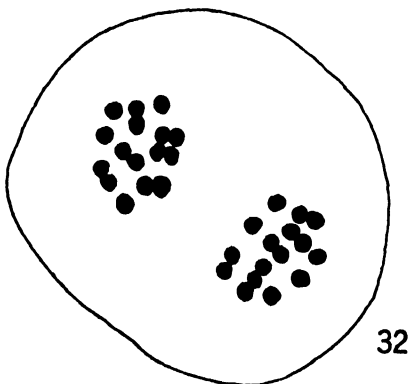
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FIGS. 26-32.—Figs. 26-28, *Myrica cerifera*: 26, first anaphase plates; 27, interkinesis; 28, polar view of second metaphase. Figs. 29, 30, *M. pumila*: 29, late diakinesis; 30, first metaphase plate. Fig. 31, *M. carolinensis*: second metaphase. Fig. 32, *Comptonia peregrina*: first anaphase plate showing secondary pairing of bivalents.

do not form. When the nuclei are completely reorganized they have withdrawn as far as possible from one another and lie close to the plasma membrane. A furrow begins in each depressed area of the mother cell and these furrows extend centripetally in such a manner that the tetranucleate protoplast is organized into four microspores, each with a single nucleus. The four young microspores are held together until the spores develop their own cell walls. After the appearance of a cell wall about each individual spore the mother cell wall begins to disintegrate, and with its disappearance the microspores are set free in the locules of the anther. Each young microspore is surrounded by a perinuclear zone of granules (fig. 40). The microspore grows rapidly and the nucleus develops a typical reticulum.

#### OTHER MYRICACEAE

Comparative studies of *M. cerifera*, *M. carolinensis*, *M. pumila*, and *Comptonia peregrina* indicate a close similarity in chromosome behavior. In all forms studied the meiotic divisions progress regularly.

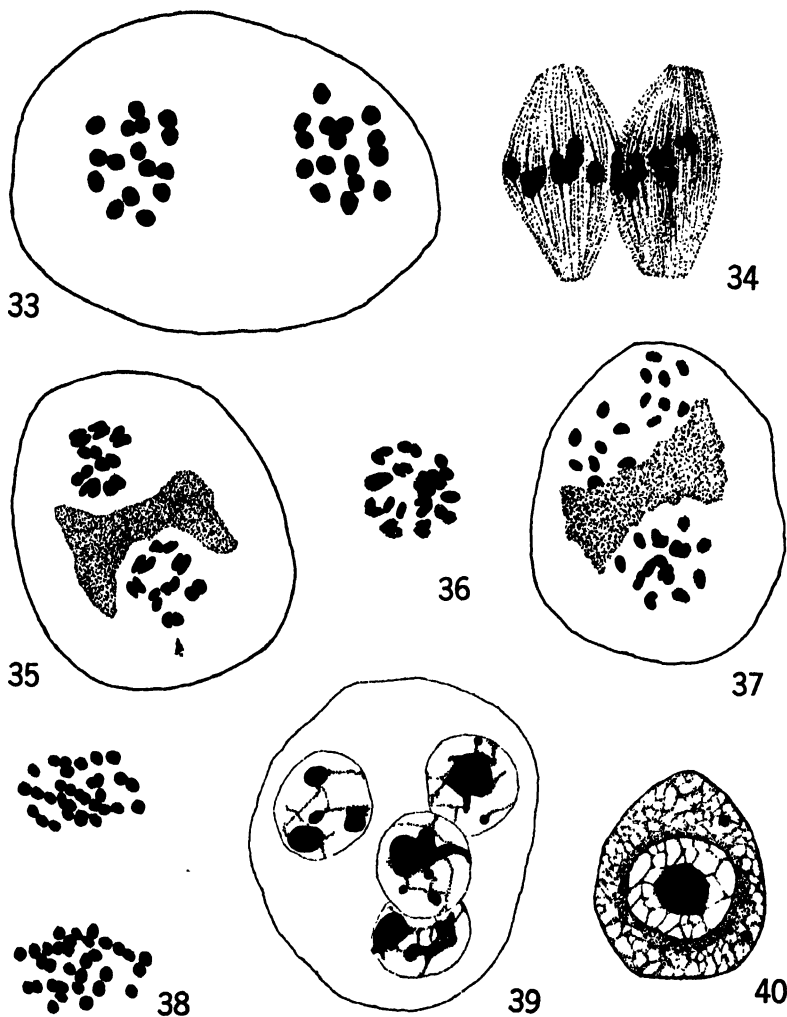
##### *Myrica carolinensis*

Examination of the meiotic processes in *M. carolinensis* reveals no outstanding differences from those described for *M. cerifera*. The same types of chromosome configurations are observable at diakinesis (figs. 14, 16). At diakinesis eight bivalents are evident and polar views of the first anaphase plate confirm this number (fig. 31). The chromosomes are characteristically short and thick and about as broad as long.

The plants used in this study and here referred to as *M. carolinensis* were thought originally to be a hybrid between *M. cerifera* and *M. carolinensis*. Meiotic studies showed no irregularities in chromosome behavior and no apparent disharmony between maternal and paternal chromosomes. This species is known to be variable in leaf size and pubescence.

##### *Myrica pumila*

The meiotic processes in *M. pumila* agree closely with those described for *M. cerifera* and for *M. carolinensis*. The same types of chromosome configuration described for *M. cerifera* are present at



FIGS. 33-40.—Fig. 33, *Comptonia peregrina*: first anaphase plate showing secondary pairing of bivalents. Figs. 34-37, *Myrica cerifera*: 34, spindles of second meiotic division; chromosomes in second metaphase; 35, 36, second metaphase stages; 37, second anaphase plates showing eight chromosomes in each plate. Fig. 38, *C. peregrina*: second anaphase plates; sixteen chromosomes in each plate, Figs. 39, 40, *M. cerifera*: 39 tetranucleate protoplast; 40, microspores.



diakinesis (figs. 17, 18). The number of bivalents is eight (fig. 19). At the first metaphase plate the eight bivalents show no recognizable differences in size (fig. 30).

### *Comptonia peregrina*

*Comptonia peregrina* was formerly described as *Myrica asplenifolia* L. The general meiotic processes in *Comptonia* agree rather closely with those described for *M. cerifera*, differing mainly in the number of chromosomes. The bivalents pass to the equator and disjoin in an orderly manner (fig. 24). Lateral views of the first metaphases and polar views of the first anaphases establish the haploid chromosome number as sixteen. Polar views of the second anaphases also show sixteen (fig. 38).

Examination of the first metaphase, first anaphase, and second anaphases gives evidence of secondary pairing (figs. 32, 33). Different bivalents are closely associated but are not in contact. The relation of secondary pairing to the homology of the associated bivalents was recognized by DARLINGTON (2). On the evidences of secondary pairing and of chromosome number it seems probable that *Comptonia peregrina* is a tetraploid relative of the *Myrica* species studied.

Incidentally SUGIURA (5) found eight as the haploid number in *Myrica rubra* also.

### Summary

1. The meiotic processes in the microspore mother cells of *Myrica cerifera* L., *M. pumila* Michx., *M. carolinensis* Mill., and *Comptonia peregrina* (L.) Coulter are described.

2. The Myricaceae range from polygamism through monoecism to dioecism. All variations observed were in the direction of changes from carpellate to staminate.

3. Material of *Myrica* and *Comptonia* was unsuitable for detailed studies on prophase stages, including chiasma formation. Synzesis is a constant feature in the species studied, whether an artifact or not.

4. Chiasmata become nearly or completely terminalized in late prophases.

5. At interkinesis the chromosomes are indistinguishable and cytokinesis does not occur at this stage.

6. Cytokinesis is by furrowing of the plasma membrane from the periphery, usually resulting in quadripartition with a tetrahedral arrangement of the microspores.

7. No evidence for the presence of heterochromosomes was observed in the meiotic divisions of any of the species studied.

8. *Comptonia peregrina* shows secondary pairing among its bivalents. This plant is probably a tetraploid relative of the *Myrica* species studied.

9. The haploid number of chromosomes is eight in *M. cerifera*, *M. pumila*, and in *M. carolinensis*, and sixteen in *Comptonia peregrina*.

The writer expresses his appreciation to Professor J. M. BEAL for the assistance given him during the course of this investigation, and to Professor C. E. ALLEN for suggesting the problem.

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# CYTOLOGICAL STUDIES IN THE GENUS PHOENIX<sup>1</sup>

## CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 486

J. M. BEAL

(WITH FIFTEEN FIGURES)

### Introduction

The date palm, *Phoenix dactylifera* Linn., was introduced into Florida and California nearly two centuries ago by Spanish explorers and missionaries. No really serious experimental work was done on this plant in this country until about the beginning of the present century, when the first successful importation of offshoots of standard date varieties was made in the summer of 1900 by the United States Department of Agriculture in cooperation with the University of Arizona. Shortly afterward two experimental date gardens were established in the Coachella Valley of California, one at Mecca in 1904 and the other at Indio in 1907. A great number of the best date varieties have since been introduced and tested in these gardens, and the growing of dates on a commercial scale is now a well established industry in certain areas.

A number of other species of *Phoenix* have also been introduced into the United States and several of these are grown in certain regions which have a suitable climate. In experiments at the Indio date garden, successful crosses have been made by using the pollen from *P. canariensis* Hort., *P. reclinata* Jacq., *P. sylvestris* Roxbg., *P. roebelenii* O'Brien, and *P. rupicola* T. Anders. on *P. dactylifera* Linn. (3). It has been shown that pollen from some of these species, as well as that from certain varieties of *P. dactylifera*, may affect not only the size, shape, and color of the seed, but also the size and time of ripening of the fruit itself. The direct effects on fruit characteristics have been termed "metaxenia" by SWINGLE (5), and its study has been continued by NIXON (2, 3).

Because of the ease with which certain species crosses may be

<sup>1</sup> This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

made, as well as the occurrence of metaxenia, a cytological investigation of some of the species and varieties used in this work has been undertaken. The microsporocytes possess small nuclei with small and numerous chromosomes which do not lend themselves to a detailed analysis of prophasic conditions, nor to a critical comparison of the range of size and form variations among the chromosomes. Hence the chief purpose of this study is to report the numbers of the chromosomes which have been found in the forms examined.

In spite of the importance of the date palm and its long history, only one reference has been found which deals with its cytology. NEMEC (1) examined young embryos of *P. dactylifera* and reported the presence of 28 chromosomes. Several species in other genera in different tribes of the Palmaceae have been investigated and chromosome numbers reported, but in only one of those examined, *Trachecarpus excelsa* Wendl. var. *fortunei* Mak. of the tribe Corypheeae (4), has the same number ( $n = 18$ ) been reported as that herein recorded for the species of *Phoenix*. The probable occurrence of an unequal pair of chromosomes was also reported for *Trachecarpus*, but my observations on three species of *Phoenix* have shown no indication of heterochromosomes.

### Material and method

Seeds of the species and varieties used for the study of mitosis were supplied by Mr. NIXON in the autumn of 1936 and the winter of 1937. The seeds were planted in sand in large pots in the greenhouses of the University of Chicago and very satisfactory germination was obtained. When the primary root had reached a length of 6–8 inches, numerous lateral roots had developed, ranging from 0.25 to 0.75 inch in length. Tips from both primary and lateral roots were fixed in Navashin's solution and in 2BE. The material was then passed through the usual schedule and imbedded in paraffin. The roots were cut transversely at  $15\ \mu$  and stained according to the gentian violet-iodine method.

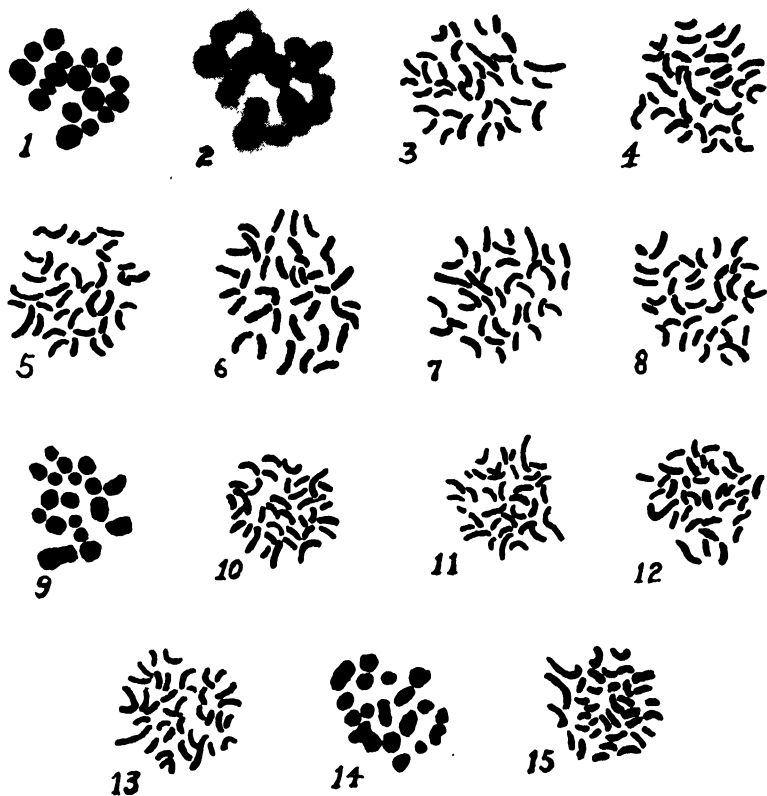
Meiotic material was secured from staminate palms growing in the U.S. Experiment Date Garden at Indio, California, and from the private gardens of a few growers nearby, in January 1937. First metaphase stages were usually present when the staminate inflores-

cences had emerged to a visible distance of 6-8 inches from the crown of leaves among which they develop. The top portions of such inflorescences with their inclosed flowers were cut off, and anthers from some of these were smeared in aceto-carmine. When desired stages were found by this method other slides were smeared immediately, fixed in Navashin's solution, and stained in gentian violet-iodine. Still other flowers from the same inflorescence were split lengthwise so as to expose the anthers, and then dropped immediately into Navashin's solution. These were later imbedded in paraffin, sectioned, and stained in gentian violet-iodine. Satisfactory preparations for study were obtained after both the smear and paraffin methods.

### Observations and discussion

The species of *Phoenix* are dioecious, and all material for the study of meiosis has been taken from staminate plants, the flowers of which generally produce six stamens. The anthers are approximately 2 mm. in length when the first meiotic metaphases occur, and they contain a fairly large number of microsporocytes. Although comparatively small, these cells separate readily in smearing. Their nuclei stain well but the chromosomes are so small and so numerous that it has not been possible to make a critical study of the prophases leading to the first meiotic division; but from the limited observations made it appears that pairing is regular, and this is confirmed by the regularly paired condition of the bivalents as observed at first metaphases. At late diakinesis the two components of each bivalent are clearly visible, while at first metaphases they are often so closely associated that they appear to consist of a single element. Still many of the bivalents show the two units of which they consist. Counts made at first metaphases show 18 pairs of chromosomes in *P. dactylifera* (figs. 1, 2), *P. canariensis* (fig. 9), and *P. sylvestris* (fig. 14). These figures also show a marked similarity in the range of size and form variations among the bivalents in the three species. Examination of first anaphases has shown regular disjunction with no signs of lagging chromosomes. Additional drawings have been made from the species and varieties used, but since they agree in number of chromosomes and other essential details with those just listed, they have not been included.

Mitotic metaphases have been studied in root tips of six species of *Phoenix* and in six varieties of *P. dactylifera*. All of these show the same number of chromosomes and the same general range of size



FIGS. 1-15.\*—Figs. 1-8, *Phoenix dactylifera*: fig. 1, Pyramid Garden, metaphase I; fig. 2, photomicrograph of same nucleus as in fig. 1. Root tip metaphase of: fig. 3, Ammary; fig. 4, Barhee; fig. 5, Deglet Noor; fig. 6, Halawy; fig. 7, Kustawy; fig. 8, Maktum. Fig. 9, *P. canariensis*, metaphase I. Root tip metaphase of: fig. 10, *P. canariensis*; fig. 11, *P. hanceana* var. *formosanum*; fig. 12, *P. humilis*; fig. 13, *P. reclinata*. Fig. 14, *P. sylvestris*, metaphase I. Fig. 15, *P. sylvestris*, root tip metaphase.

\* All figures approximately 2450X, except figure 2, a photomicrograph, which is about 3300X.

and form variations. Especially worthy of note is the marked similarity shown in the somatic complements of the six varieties of *P. dactylifera* (figs. 3-8). Four or five pairs of chromosomes are

longer than the remainder, and this size variation agrees with that observed in the meiotic metaphases.

Metaphases from root tips of *P. canariensis* (fig. 10), *P. hanceana* var. *formosanum* (fig. 11), *P. humilis* (fig. 12), *P. reclinata* (fig. 13), and *P. sylvestris* (fig. 15) show essentially the same range in size and form variations as do those of *P. dactylifera* varieties. The chromosomes in these species are perhaps slightly smaller on the whole, but this seems not to be a matter of any special significance, since certain of them have been used successfully as pollen parents in crosses with *P. dactylifera*.

TABLE 1

VARIETY	CHROMOSOME NUMBERS	
	n	2n
<i>P. canariensis</i> .....	18	36
<i>P. dactylifera</i> var. Ammary .....		36
Barhee .....		36
Deglet Noor .....		36
Fard no. 4 .....	18	
Halawy .....		36
Kustawy .....		36
Maktum .....	18	36
Menakher .....	18	
Mosque .....	18	
Pyramid Garden .....	18	
<i>P. hanceana</i> var. <i>formosanum</i> .....		36
<i>P. humilis</i> .....		36
<i>P. reclinata</i> .....		36
<i>P. sylvestris</i> .....	18	36

Table 1 gives the complete list of the species and varieties examined, together with the observed numbers of their chromosomes.

The haploid number of chromosomes has been determined from staminate plants which were imported as offshoots, from plants propagated as offshoots from the original importations, or from plants derived from seeds of named varieties. The following excerpt from a letter written by Mr. NIXON to me gives some interesting facts bearing upon this matter, together with some equally interesting occurrences in certain carpellate plants:

"There are only a few imported male palms in this country. While some few offshoots of these have been propagated, nearly all grow-

ers depend on males grown from seed planted here or offshoots therefrom. . . . Strictly speaking there is no such thing as a 'Deglet Noor' male or 'Barhee' male. Though sometimes referred to as such, it is generally understood that what is meant is a seedling grown from seed of the variety named. We have at the station here one male which is three-quarters Deglet Noor, or at least it was grown from seed that was pollinated with pollen from a Deglet Noor seedling male. However, for most of the males around the Coachella Valley little is known of the pollen parent and, of course, what complicates the matter still further genetically is that we know nothing of the pollen parent of any of the imported female varieties, and the presumption is that nearly all of them were originally seedlings.

"In any large planting of date seeds there will be approximately equal numbers of staminate and carpellate plants. It is not uncommon for staminate flowers to have carpels. Of about 70 male palms formerly growing at this station approximately one-fourth of them were observed to have occasionally flowers with carpels—such flowers occurring usually on the smaller and later inflorescences and commonly being clustered near the basal (proximal) part of the strand or branchlet. Usually these carpels make a very slight growth, just enough to be conspicuous late in the season and to keep the flower stalk from drying up. Very, very rarely some of these carpels will take pollen and develop into a date with a seed. I had heard of a few such instances, but had never seen a male bearing dates with seed until two years ago when my attention was called to *five* males each bearing a few dates with seed in a seedling planting at the University of Arizona Date Garden near Tempe, Arizona. I have never seen a female palm having flowers with stamens, but as it would be much easier to overlook stamens than carpels it may be that hermaphroditic flowers do occasionally occur on female palms. Incidentally, I understand that Prof. W. E. BRYAN, plant breeder at the University of Arizona, is having the seed from male palms mentioned above planted.

"Some of the seedling males that are being propagated now by offshoots have been given names and in time may acquire the status



of varieties. In fact, the only distinction between seedlings and varieties is that the former are grown from seed and the latter from offshoots."

It is generally known to date growers that the seedlings of a given variety do not "come true" to that variety in the type of fruits produced. The staminate seedlings appear to be equally variable, and there is probably as great variation between staminate and carpellate progeny as there is among the carpellate seedlings.

Since all the studies on mitosis reported here were made on root tips obtained from seedlings grown from seeds of named species or varieties, and since the chromosome complements show such marked similarity, the variability of the seedlings (whether carpellate or staminate) is probably due to differences in genetic factors rather than to structural changes in the corresponding or homologous chromosomes. Interfertility among the species may be explainable on the basis of similar form and like number of chromosomes in which perhaps little change (such as might affect their ability to pair as homologous chromosomes) has occurred, even though there may be much variation in size and vegetative characters as between different species.

### Summary

Meiotic metaphases of *P. dactylifera*, *P. canariensis*, and *P. sylvestris* show 18 bivalent chromosomes in microsporocytes. Mitotic metaphases of root tip cells in six varieties of *P. dactylifera*, in *P. canariensis*, *P. hanceana* var. *formosanum*, *P. humilis*, *P. reclinata*, and *P. sylvestris* show 36 chromosomes. There is marked similarity in the range of size and form variations among the chromosomes in all species and varieties examined. This perhaps accounts for the interspecific fertility which has been demonstrated through crosses made between several of the species.

The writer expresses his appreciation to Dr. E. C. AUCHTER, U.S. Bureau of Plant Industry, for permission to use the facilities of the U.S. Experiment Date Garden at Indio, California; to Mr. FRANK

THACKERY for placing at his disposal the laboratory and palms growing at the garden; and to Mr. ROY W. NIXON for supplying the seeds used and for assistance in collecting the meiotic material.

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## ACTIVITY OF THE POTASSIUM SALT OF INDOLE(3)ACETIC ACID IN THE AVENA TEST

DAVID M. BONNER

AVERY, BURKHOLDER, and CREIGHTON (1) have presented data which indicate that the potassium salt of indole(3)acetic acid possesses an activity higher than that of the free acid. This same observation has been made by ZIMMERMAN and HITCHCOCK (3) concerning the potassium salt of naphthaleneacetic acid. Under certain conditions they found the magnitude of this increase to be nearly as great as twice the activity of the free acid. AVERY, BURKHOLDER, and CREIGHTON state that this is apparently a salt effect, and not a pH effect, since they adjusted the pH of the agar blocks to various values and found no difference in activity between a pH of 6.6 and one of 3.0. This later observation directly contradicts their earlier statement that potassium indole(3)acetate is more active than indole(3)acetic acid; that is, at pH 6.6 the solution consists of 98% salt, and at pH 3.0 of 98% free acid. The method they used in obtaining the desired pH was not stated, and possibly buffered solutions were not used.

From theoretical considerations a strong salt effect and no pH effect would be unlikely, and contradictory to data obtained in this laboratory. The first most obvious difference in composition between a solution of the salt of indole(3)acetic acid and one of the free acid itself is the presence of potassium ion in the case of the solution of the salt, and its absence in the case of the solution of the free acid. One would hardly expect, however, the presence of a trace of an inorganic ion, already present in comparatively high concentration in the cell, to make a significant difference in the activity of the substance. The salt when dissolved hydrolyzes to some extent, forming free indole(3)acetic acid and hydroxyl ion. The concentration of the salt that was used was very low, and so, as was seen from actual measurement as well as from calculations, the final pH was 6.2. The free acid on the other hand undergoes

only dissociation when dissolved in water. Since the acid is a comparatively weak one ( $pK = 4.75$ ) the amount of dissociation is small, but is sufficient to make the pH of the solution about 5.5. Thus the principal difference between the two solutions is that, whereas that of the salt contains principally ions (potassium ion and indole(3)acetate ion) that of the acid contains to a large extent molecules of the free acid. Since J. BONNER (2) has already shown that for optimal activity the growth substance must be present in the associated form, one would expect that for unbuffered solutions of equivalent concentrations the free acid would possess the greater activity.

The crystalline salt was prepared from the indole(3)acetic acid of Merck. As a check, a sodium salt prepared by Dr. J. B. KOEPFLI was also used. The salts and the free acid were tested by the standard *Avena* test, a technique which differs only slightly from that used by AVERY, BURKHOLDER, and CREIGHTON, who used only one decapitation and photographed the plants two hours after application of the blocks. The three substances were tested in buffered and unbuffered solutions. The buffered solution used was a mixture of potassium dihydrogen phosphate and disodium hydrogen phosphate, thereby eliminating the lack of potassium ion in the case of the free acid. All hydrogen ion concentrations were measured by means of a glass electrode. The concentrations were the same as those which other workers had found to exhibit a very marked increase of activity of the salt over that of the free acid.

Table 1 gives the data obtained from several independent experiments. From these data certain points are clear. The activity of the salt was not found to be greater than that of the free acid; in fact if equal weights of the three substances are considered, the activities of the salts were consistently lower than that of the free acid, the activity of the potassium salt being lowest. In the case of the unbuffered solutions the difference in activity is very great, the salts being much less active than the free acid. With the application of the correction for the difference in molecular weights, the differences of activity in unbuffered solutions are smaller, and it is probable that this difference is a pH effect; that is, it is due to the higher pH of the salt solutions. Evidence that this is a pH effect is given

by the change in relative activities when the solutions are buffered to the same pH. Buffering at pH 6.05 gave an increase in the activities of the two salts relative to their activities in unbuffered solutions, whereas the activity of the free acid, although increased, was much less than that of the salts. Here again a correction for the

TABLE 1

SUBSTANCE	CONC. MG./L.	pH	TIME BE- TWEEN DECAPITATIONS (HOURS)	NO. OF PLANTS	AVERAGE CURVA- TURE	CURVA- TURE COR- RECTED TO SAME MOLALITY
Indole(3)acetic acid.....	0.12	6.05	3.5	12	15.3	15.3
Potassium indole(3)acetate..	"	"	"	11	12.7	15.4
Sodium indole(3)acetate...	"	"	"	12	13.7	15.4
Indole(3)acetic acid.....	"	"	4.0	11	16.8	16.8
Potassium indole(3)acetate..	"	"	"	10	12.5	15.2
Sodium indole(3)acetate...	"	"	"	11	14.3	16.1
Indole(3)acetic acid.....	"	"	3.0	12	17.8	17.8
Potassium indole(3)acetate..	"	"	"	12	13.3	16.2
Sodium indole(3)acetate...	"	"	"	12	15.1	17.0
Indole(3)acetic acid.....	0.09	Unbuf- fered	"	18	9.7	9.7
Potassium indole(3)acetate..	"	"	"	24	2.8	3.4
Sodium indole(3)acetate...	"	"	"	24	3.7	4.1
Indole(3)acetic acid.....	"	6.05	"	22	16.9	16.9
Potassium indole(3)acetate..	"	"	"	18	14.1	17.3
Sodium indole(3)acetate...	"	"	"	24	15.4	17.3

differences in molecular weights must be applied. If this is done, the activities of the three substances are found to be identical within experimental error.

### Summary

The results obtained by various workers which seem to show a greater activity of the potassium salt of indole(3)acetic acid in comparison with that of the free acid were tested. In several experiments it was found that the three substances indole(3)acetic acid, potassium indole(3)acetate, and sodium indole(3)acetate possessed similar activities when their equimolar solutions, buffered at the same pH, were given the *Avena* test. Thus, as would be expected from theoretical considerations, these three substances, if

buffered at the same pH, possess the same activity. In the case of the unbuffered solutions it is suggested that the difference in activity may be due to a pH effect.

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# RESPONSES BY TOMATO PLANTS TO ARTIFICIAL ILLUMINATION<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 487

JOHN W. MITCHELL

(WITH TWO FIGURES)

## Introduction

It has been shown (6, 7, 8, 9) that under known environmental conditions some plants grown in filtered sunlight exhibit characteristics peculiar to that part of the spectrum with which they are illuminated. In general, it was found that plants illuminated with light especially rich in blue and violet grew less in height per unit of time, but synthesized more solid matter, than did similar plants grown in an equal intensity of light rich in red, orange, and yellow.

Similar results were observed by DASTUR (1), using plants grown under artificial illumination. He found that, with equal intensities of total radiant energy, the amount of carbohydrate synthesized by leaves of plants illuminated by means of a carbon arc (rich in blue and violet) was greater per unit of time than that synthesized by leaves illuminated by means of a gas filled electric lamp (rich in yellow, red, and infra-red).

The growth responses and carbohydrate content of tomato plants, illuminated by means of artificial light from sources similar to those used by DASTUR, have been studied under controlled environmental conditions in the present investigation.

## Material and method

Tomato plants (*Lycopersicum esculentum*) were grown in fertile soil contained in 6 inch clay pots under conditions that prevailed in the greenhouses during the spring and early summer. Sixty-five to one hundred uniform plants were selected after they had attained a height of approximately 8 inches. They were divided into three

<sup>1</sup> This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

groups of equal numbers. The first group was harvested immediately and designated initial controls; the remaining two groups were placed in different rooms and grown under controlled environmental conditions with the use of artificial illumination (4).

The temperature in both rooms varied between 68° and 71° F. during periods of illumination and 62° to 65° F. during periods of darkness. The humidity varied between 50 and 60 per cent of saturation. Plants in both rooms were illuminated 14 hours daily.

A 2000 watt Mazda lamp was used as a source of illumination in one room, while a 3000 watt (50 volt 60 ampere) carbon arc supplied light in the other. "Sunshine" carbons were used in the arc lamp, and the light was filtered through ordinary window glass.

Two experiments were conducted in which the light intensities were balanced by different means. In the first the plants were so arranged that the intensity of total radiant energy from the two sources was equal when measured at the surface of the leaves by means of a thermopile. In the second the plants were so arranged that they received equal intensities of light from the respective sources, as measured by means of a Weston photronic cell.

**DRY WEIGHT AND CARBOHYDRATE DETERMINATIONS.**--After the plants had grown for several weeks under controlled conditions, they were washed free from soil and divided into three fractions, roots, stems, and leaves. Any leaves that fell from the plants during the experiment were added to the final leaf samples. These fractions were cut finely and the fresh weight determined. They were then dried for 24 hours in a well ventilated oven and the dry weight determined by weighing in large weighing bottles on an analytical balance.

The samples were prepared for analysis by first grinding in a coffee mill and then in a ball mill until the powder could be brushed through an 80 mesh screen. After redrying in partial vacuum at 80° C., duplicate samples of the various fractions were hydrolyzed by boiling for 2½ hours with 2.5 per cent HCl. The acid was neutralized with Na<sub>2</sub>CO<sub>3</sub>, and the solutions cleared as described by LOOMIS and SHULL (3). The reducing power of the final solutions was then determined by the method described by PHILLIPS (5), and expressed



in terms of glucose. This fraction, consisting of sugars, starch, dex-  
trins, hemicelluloses, and other materials that hydrolyzed to yield  
sugar under these conditions, is designated total carbohydrate.

### Results and discussion

As shown in figure 1, the radiant energy emitted by the arc was especially rich in blue and violet light, with a relatively low intensity of yellow, orange, red, and infra-red. In contrast to this, radiant energy emitted by the Mazda lamp was especially rich in yellow, orange, red, and infra-red, with a relatively low intensity of blue and violet.

Recent work (2) has shown that the entire visible spectrum is effective in photosynthesis. Although the Weston cell is particularly sensitive to visible light, it is not an entirely satisfactory means of estimating energy in this region of the spectrum, as it is not equally sensitive to all wave lengths (fig. 2). This cell is highly sensitive to green, yellow, and orange light, and its sensitivity decreases with wave lengths either longer than approximately 630 or shorter than approximately 530 milli-microns. Furthermore, the sensitivity of this cell does not parallel the effectiveness of the different wave lengths in photosynthesis (2).

A thermopile is likewise not an entirely satisfactory means of comparing the intensities of light from different sources. Although it is equally sensitive to light that produces photosynthesis, it is also sensitive to wave lengths which are not essential to the production of solid matter by plants.

RESPONSES TO EQUAL INTENSITIES OF TOTAL RADIANT ENERGY.—Under conditions of equal total radiation, the intensity of illumination from each source was equivalent to 0.157 gram calories per square centimeter per second, as measured by the thermopile. From 30 to 35 per cent of the energy radiated by the arc was of wave lengths between 4000 and 7000 Å.u., while only 15 to 20 per cent of the energy radiated from the incandescent lamp fell within this region of the spectrum.

Under these conditions, the stems of plants illuminated with light from the arc lamp elongated less during a period of two weeks and developed narrower and thicker leaves, which were lighter green in

color, than did similar plants illuminated with the same total intensity of light from the Mazda lamp.

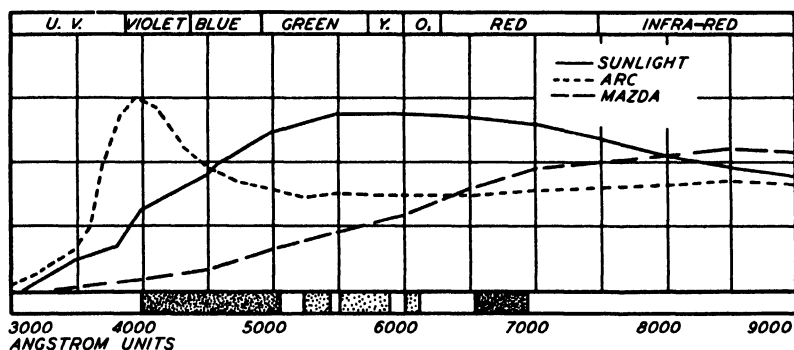


FIG. 1.—Spectral energy distribution of light from different sources. Each square in curve illustrating sunlight represents 2500 micro-watts of radiant flux per square centimeter. Each square in curves illustrating the arc and 1500 watt incandescent lamp represents 250 and 25 micro-watts per square centimeter respectively, at 1 meter distance from source. Approximate wave lengths of visible light absorbed by chlorophyll shown by shading along base line.

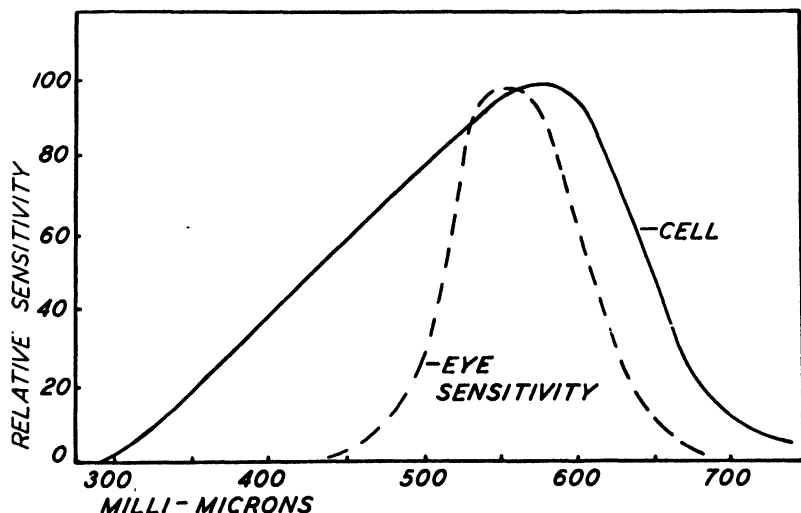


FIG. 2.—Relative sensitivity of photronic cell, average relative current output for equal energy rates, or radiant flux, at various wave lengths. Standard eye sensitivity curve given for comparison.

On the basis of one hundred plants, those illuminated by means of the arc synthesized more than twice as much dry matter and four

times as much total carbohydrate during a period of two weeks than did plants illuminated by means of a Mazda lamp for the same period of time (tables 1 and 2). The differences in gain in dry weight

TABLE 1

COMPARISON OF GAIN IN DRY MATTER DURING TWO WEEKS BY TOMATO PLANTS GROWN IN LIGHT EMITTED BY AN INCANDESCENT LAMP AND BY OTHERS ILLUMINATED BY MEANS OF AN ARC LAMP, EXPRESSED AS GRAMS DRY MATTER PER 100 PLANTS

REGION	LIGHT BALANCED BY THERMOPILE		LIGHT BALANCED BY PHOTOCELL	
	MAZDA	ARC	MAZDA	ARC
Roots.....	8.36	31.20	30.35	38.12
Stems.....	23.97	30.80	66.25	49.31
Leaves.....	43.58	112.37	83.68	71.55
Total.....	75.91	174.37	180.28	158.98

TABLE 2

COMPARISON OF GAIN IN TOTAL CARBOHYDRATES DURING TWO WEEKS BY TOMATO PLANTS GROWN IN LIGHT EMITTED BY AN INCANDESCENT LAMP AND OTHERS ILLUMINATED BY MEANS OF AN ARC LAMP, EXPRESSED AS GRAMS PER 100 PLANTS

REGION	LIGHT BALANCED BY THERMOPILE		LIGHT BALANCED BY PHOTOCELL	
	MAZDA	ARC	MAZDA	ARC
Roots.....	1.04	4.82	.....	.....
Stems.....	3.69	6.69	.....	.....
Stems and roots.....	4.73	11.51	19.34*	14.84
Leaves.....	5.06	27.33	23.09	16.61
Total.....	9.79	38.84	42.43	31.45

\* Stem and root tissue combined to obtain sufficient sample for analysis.

and carbohydrate content were especially great in the roots and leaves.

Thus when the intensity of total radiant energy from the two sources was made equal, as measured by means of a thermopile, the

arc actually radiated a greater intensity of those wave lengths which were effective in the synthesis of solid matter, and as a result the plants illuminated by means of the arc synthesized the greater quantity of solid materials per unit of time (fig. 1). Calculated on the basis of energy consumption, one hundred plants illuminated by means of the arc gained 0.282 gm. per kilowatt hour while those illuminated by means of the incandescent lamp synthesized only 0.184 gm. per kilowatt hour, when the incident energy from the two sources was equal at the surfaces of the leaves.

RESPONSES TO EQUAL INTENSITIES OF LIGHT TO WHICH A PHOTRONIC CELL IS SENSITIVE.—The plants were so arranged that the intensity of those wave lengths of light, to which a Weston photronic cell is sensitive, were made equal to 700–800 foot candles in the case of illumination from both the arc and the incandescent lamp. Under these conditions the stems of plants illuminated by means of the incandescent lamp grew more in length, and the leaves were a lighter green color and thinner than those of plants illuminated by means of the arc. The difference in length of internodes, however, was greatest during the second week of illumination and became less noticeable during the third week.

Good growth was evidenced in those plants illuminated by means of the incandescent lamp and also those illuminated by means of the arc, as they increased in dry weight per one hundred plants from approximately 111 to 343 gm. and 111 to 316 gm. respectively. Plants grown with the aid of the incandescent lamp, however, gained slightly more in dry matter, and approximately 30 per cent more in total carbohydrates, during a period of two weeks, than did similar plants exposed for the same length of time to illumination from the arc (tables 1 and 2). Microchemical tests made at the end of the experiment likewise showed that the most starch had been synthesized by plants grown with the aid of the Mazda lamp. Interpretation of these differences in dry weight cannot be attempted without more detailed experiments.

It is concluded from the data presented, first, that under conditions of equal total radiant energy a much greater increase in dry weight was made per unit of time, and per unit of electrical energy consumed, by plants illuminated by means of a carbon arc than by

plants illuminated by means of an incandescent lamp, under the same conditions. This result was to be expected, since those wave lengths of light utilized by plants in the synthesis of solid matter represent a considerable portion of the radiant energy emitted by the arc, while light of a similar quality represented a smaller part of the total radiant energy emitted by the incandescent lamp. Second, it is evident that when light from the two sources was balanced by means of a photronic cell, the plants synthesized more nearly the same amount of solid matter. Through the use of this cell, the intensities of those wave lengths of light from the two sources that were essential to the production of dry matter were made more nearly equal than when the incident energy was balanced by means of a thermopile. Although the photocell was not entirely satisfactory as a means of equalizing incident energy from the two sources, it gave a more reliable measure of those wave lengths of light concerned in the growth of plants than was obtained through the use of a thermopile.

### Summary

1. Tomato plants grown in the greenhouse were transferred to controlled environmental conditions. Some were illuminated daily by means of a carbon arc, while others were grown under the same conditions but illuminated by means of an incandescent lamp.

2. The anatomical responses and carbohydrate content of the plants were studied, first, when illuminated with an equal intensity of total radiant energy from the two sources; second, when illuminated with an equal intensity of those wave lengths to which a photronic cell is sensitive.

3. When plants were supplied with equal intensities of total radiant energy from the two sources, those illuminated by means of the arc grew less in height and synthesized more than twice as much solid matter, and approximately four times as much acid hydrolyzable materials and sugars during two weeks, than did plants that were illuminated by means of the incandescent lamp for the same length of time. On the basis of electrical energy consumption, the arc was approximately 53 per cent more efficient in stimulating the production of dry matter than was the incandescent lamp, when there was equal incident energy at the leaf surfaces. This difference

in the rate of production of solid matter, under conditions of equal total radiant energy, was possibly due to the fact that there was actually radiated from the arc a greater intensity of those wave lengths of light which are known to accelerate the process of photosynthesis.

4. An appreciable and more nearly equal gain in dry weight and carbohydrate content was evidenced both in those plants illuminated by means of the arc and also those illuminated by means of the incandescent lamp, when light from the two sources was balanced by means of a photronic cell. Under these conditions, plants illuminated by means of the arc again developed shorter and thicker stems, but synthesized slightly less solid matter per unit of time than did plants illuminated by means of the incandescent lamp.

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# DAILY PERIODICITY OF STOMATA IN CERTAIN SPECIES OF TURF GRASSES

J. C. CARROLL AND F. A. WELTON

## Introduction

In connection with certain physico-chemical determinations on several species of turf grasses, a limited study of their stomatal behavior was included. Observations were made on *Poa pratensis*, *P. trivialis*, *Agrostis alba*, *A. tenuis*, and *Festuca rubra fallax*. These grasses were grown on Wooster silt loam soil in the summer of 1931, and maintained under lawn conditions. Data were secured relating to the number, size, and periodicity of their stomata.

The general structural characteristics of the stomatal apparatus of any group of plants seem to be peculiar to that group, and according to SCHWENDENER (6) and REHFOUS (4) are of first importance in indicating phylogeny and relationships. The number of stomata per unit of leaf surface is, within certain limits, a characteristic of the particular species or variety of plant under consideration.

MILLER (3) observed the stomata of various agricultural plants for several growing seasons and found that the number per unit of surface area in a given species differed from season to season. Evidence as to the effect of environmental conditions on the number of stomata is somewhat contradictory. A high humidity, however, apparently exerts considerable influence in reducing the stomatal frequency. SALISBURY (5), in a study of the stomatal frequency of woodland flora, noted a higher frequency under dry exposed conditions than under wet, and found a high positive correlation between the number of stomata and the number of epidermal cells per unit of area. He proposed the stomatal index, which expresses the proportion, in percentage, of the ultimate divisions of the dermatogen of the leaf which have been converted into stomata. He considered variations in the stomatal index to be due to internal factors, of which nutritional conditions are perhaps the most decisive.

BLAYDES (1) found that the young leaves on a number of plants

possessed no stomata, whereas mature leaves on the same plants showed large numbers per unit area.

The method of investigation used in this study was the one suggested by LLOYD (2), in which pieces of epidermis were removed and immediately placed in absolute alcohol. Preliminary observations showed the stomata in all the species examined to be more numerous near the tip than near the base of the blade. Observations on greenhouse material showed that young leaves had fewer stomata per unit area than had mature leaves, and that they were usually closed. This indicated that the major part of the transpiration was cuticular. Two of the species studied, *Poa pratensis* and *Agrostis alba*, had stomata on both surfaces; the other three, on the upper surface only.

The observations recorded in tables 1 and 2 were made on the epidermis taken from mature leaves during a period of three consecutive clear days, August 27, 28, and 29, 1931. Small pieces of epidermis were removed from the middle portion of the blade between the midrib and the edge at hourly intervals over a 24-hour period. A Leitz Filar micrometer ocular and a 4-mm. objective were used. The width and length of the stomatal pore were measured in microns and are expressed as number per square millimeter. Each value represents the average reading from thirty fields of the microscope.

### Results

The stomata of all five species showed a definite periodicity, being open during the day and closed at night. The percentage of stomata open and closed at the same hour in the different species varied markedly (table 1).

The length of the stomatal pore did not change in a regular manner during the time it was open, but the values of both length and width in the different species fluctuated considerably, indicating that there was considerable variation in the size of the stomata. The width of the pore showed a definite maximum and minimum, reaching a maximum in the forenoon. The correlations between the number of stomata and epidermal cells per unit area, as expressed by the stomatal index, show very little difference among the species studied.



TABLE 1

CONDITION OF STOMATA IN FIVE SPECIES OF GRASS AT DIFFERENT HOURS OF THE DAY (AVERAGE READING OF THIRTY FIELDS)

SPECIES	CONDITION OF STOMATA	PERCENTAGE							
		6:00	8:00	10:00	12:00	2:00	4:00	6:00	8:00
		A.M.	A.M.	A.M.	M.	P.M.	P.M.	P.M.	P.M.
<i>Poa pratensis</i>	Fully open.....	0	7	49	39	32	14	0	0
	Partly open.....	40	85	49	45	59	64	42	5
	Closed.....	60	8	2	16	9	22	58	95
<i>Poa trivialis</i>	Fully open.....	8	12	63	58	38	30	0	0
	Partly open.....	84	86	37	36	57	59	60	2
	Closed.....	8	2	0	6	5	11	40	98
<i>Agrostis alba</i>	Fully open.....	0	.....	10	.....	0	.....	0	.....
	Partly open.....	15	.....	35	.....	40	.....	0	.....
	Closed.....	85	.....	55	.....	60	.....	100	.....
<i>Agrostis tenuis</i>	Fully open.....	0	.....	15	.....	12	.....	0	.....
	Partly open.....	25	.....	58	.....	45	.....	27	.....
	Closed.....	75	.....	27	.....	43	.....	73	.....
<i>Festuca rubra fallax</i>	Fully open.....	0	8	12	5	10	13	6	0
	Partly open.....	72	70	76	81	76	70	82	4
	Closed.....	28	22	12	14	14	17	12	96

TABLE 2

PERIODICITY OF STOMATA IN FIVE SPECIES OF GRASS

SPECIES	No. OF STOMATA PER SQUARE MM.		LENGTH AND WIDTH OF PORE IN MICRONS										STOMATAL INDEX
			UPPER SURFACE										
	UPPER SURFACE	LOWER SURFACE	4:00 A.M.	6:00 A.M.	8:00 A.M.	10:00 A.M.	12:00 M.	2:00 P.M.	4:00 P.M.	6:00 P.M.	8:00 P.M.		
Poa pratensis	160	104	o	14×1.1	16×2.1	17×2.4	17×2.2	13×2.2	13×1.6	13×1.5	o	28.6	
Poa trivialis...	160	o	o	18×1.0	18×1.6	14×1.7	16×1.3	16×1.1	16×0.9	14×0.7	o	24.0	
Agrostis alba	130	91	o	9×0.8	11×0.8	10×1.1	.....	11×0.6	.....	o	o	23.4	
Agrostis tenuis	140	o	o	18×1.0	18×1.7	17×1.7	15×1.7	14×1.2	15×1.1	15×0.8	o	25.2	
Festuca rubra fallax.....	110	o	o	23×1.1	21×1.6	20×2.6	24×2.0	22×2.4	19×1.8	22×1.4	o	23.2	

### Summary

1. A study was made of the number, size, and periodicity of the stomata of five species of grass.
2. The stomata of the five species showed a definite periodicity, being open during the day and closed at night.
3. The species showed considerable differences in the percentage of stomata open at the same period.
4. The fescue possessed fewer stomata per unit area than did either the bent or the bluegrass.

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## CURRENT LITERATURE

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*Flora of Southeastern Washington and of Adjacent Idaho.* By HAROLD ST. JOHN. Pullman, Washington: Students Book Corporation, 1937. Pp. 531 with 11 text figures and one colored map.

This book is one of the best of the more recent contributions to a knowledge of our country's flora. Although purporting to cover a comparatively small range, it is in reality a good-sized manual, the text proper dealing with 459 genera, 1187 species, and 286 subdivisions of species. Of the species and their subdivisions, 1266 are indigenous and 207 are adventive. The author "does not subscribe to the recognition of minute genera and species." Throughout the work he has "endeavored to accept as genera and species the so-called Linnaean genera and species, and to reduce to subdivisions of species those minor elements so frequently announced as species by recent American botanists." The text is therefore in keeping with the standards and ideals of our more conservative taxonomists.

The typographical work is exceptionally accurate. The so-called ENGLER and PRANTL system of classification is employed mainly, in preference to the systems of BESSEY and of HUTCHINSON. In general the International Rules of Botanical Nomenclature, as adopted at Cambridge and modified at Amsterdam, are followed. Much emphasis is placed upon life zones, as originally described by Dr. C. H. MERRIAM and later recognized by C. V. PIPER in his *Flora of Washington*. Thus, for example, the Upper Sonoran, Canadian, Hudsonian, etc., are shown in contrasting colors on the map that serves as a frontispiece, and the descriptions usually are followed with an abbreviation indicating the life zone or zones inhabited. Several kinds of type are employed, by which are shown whether a plant is indigenous or adventive, etc. A few simple illustrations accompany the analytical key to the families, these serving to elucidate points that sometimes are obscure or confusing to beginners in the study of plant identification.

A feature that characterizes the text throughout, as contrasted with most floral manuals, is the wealth of critical notes arising from the author's extensive field, herbarium, and library studies. They are concerned especially with the use of the plants as forage or in making drugs and medicines, occurrence of poisonous principles, data as to type specimens, etc. It is noted with regret, however, that he omits all authorities for the names of genera, an omission that can be of no advantage to serious students.

There is a comprehensive glossary, followed by an explanatory list of authors'

names, a list of new species (and new varieties) and new combinations, a small list of additions and corrections, and a carefully detailed index. The list of authors' names is nearly complete for the volume, only a few having been omitted through oversight or because of insufficient data (N. B. SANSON and JAMES CASSIDY, to name two instances). This list gives valuable data as to the full names of authors, their nationality, and the dates of their birth and perhaps death. The author acknowledges his indebtedness to the distinguished American plant bibliographer, Dr. JOHN HENDLEY BARNHART, for assistance in its compilation.—E. E. SHERFF.

*Economic Botany*. By ALBERT F. HILL. New York: McGraw-Hill Book Co., 1937. Pp. x+592. Figs. 225. 1937. \$4.00.

For many years botanists have occasionally turned their attention to economic phases of their subject and put forth treatises concerned with various aspects of the subject. The present volume is announced as a text. It treats of a long list of species and varieties of plants, some part or parts of which have been put to economic use. The handling of each form is brief, often so brief that one gains little more than the fact that the species exists and is of some use. The net result is a brief encyclopedia, of use as a general reference book.—E. J. KRAUS.

*The Design of Experiments*. By A. R. FISHER. London: Oliver & Boyd, 1937. Pp. xi+260. Figs. 5. 12. 6 net.

A second edition of this book, first published in 1935, permits the author to make some necessary numerical corrections of errors. The main changes from the first edition occur in sections 35 and 47.1. In section 35, orthogonalized squares receive slightly fuller treatment; in section 47.1, some examples have been added of combinatorial arrangements. Otherwise the work is practically the same as the first edition.—C. A. SHULL.

*Transactions of the Bose Research Institute, Calcutta*. Vol. X, 1934-1935. Biological and Physical Researches. Edited by SIR JAGADIS CHUNDER BOSE. London: Longmans, Green and Co., 1937. Pp. vi+240. Illustrated.

The introduction to this report gives a brief synopsis of the papers included in the main body of the work. Eight of the reports are of botanical interest, with subjects as follows: modifying effect of age on the physiological activities of the leaf of *Mimosa pudica*, by S. C. DAS and B. K. PALIT; the effects of continuous and of intermittent illuminations on phototropism, and the effects of continuous and of intermittent illuminations on longitudinal growth, by the same authors; investigations on the "after-ripening" of the seed, and effect of variation of temperature on the respiration of flower (*Helianthus annuus*), by B. K. DUTT and A. GUHA THAKURTA; chemical examination of the Indian

medicinal plant *Trichosanthes dioeca*, by N. C. NAG; examination of seeds of certain varieties of *Mecanopsis* as source of oil and manure, by N. C. NAG and H. N. BANERJEE; and chemical and physiological investigations on the presence of vitamin C in certain substances in plants, by H. N. BANERJEE.—C. A. SHULL.

*British Grasses and Their Employment in Agriculture.* By S. F. ARMSTRONG. London: Cambridge University Press, 1937. Pp. 350. Illustrated. \$5.25.

This is the third edition of a very useful work, which gives a rather detailed account of the grasses of economic importance in England, and includes descriptions of the species and their agricultural uses.

The book is divided into two parts, a botanical section and an agricultural section. In the first there are chapters on grass morphology and distribution of British grasses according to their habitats. These are followed by three keys to the more common grasses, the first based on vegetative characters, the second on floral characters (arrangement of spikelets, number of florets, etc.), and the third on "seed" characters, the seed being the entire floret composed of the lemma and palea with the included caryopsis. The last chapter in this section contains a popular description of each grass with especial emphasis on the vegetative characteristics.

The agricultural section deals primarily with the uses of each species, and their relative value as pasture or forage plants. Especial attention is given to the seed supplies, including the source, percentage of purity, and percentage of germination.

With the growing realization of the importance of grasslands and their proper use, the chapters on grassland management and improvement of poor grassland are timely and practical. Another chapter is devoted to the formation and maintenance of lawns and greens. The final chapter deals with the valuation and purchase of grass seeds, emphasizing that cheap seeds of low quality are more expensive per "real value" than good quality seeds.

The book is well illustrated with photographs and drawings, many of the latter having been reproduced from Dr. A. S. HITCHCOCK's *Manual of the Grasses of the United States*.—J. R. SWALLEN.

*Floral Morphology, a new outlook.* By E. R. SAUNDERS. Vol. I, 8 vo. Cambridge: W. Heffer and Sons, Ltd., 1937. Pp. viii+132.

For many years the author has been studying floral morphology and about thirty papers have already appeared. The present volume, which is the first of a two volume set, summarizes these papers and, giving general conclusions drawn from the entire investigation, is designed to serve as a guide in laboratory study.

The book is divided into four parts, the first dealing with the introduction and general considerations, while the other three deal with thirty-nine of the

angiosperm families. The second volume will add to the number of families. In the table of contents, the dicotyledons and monocotyledons are given as if the two groups were of equal rank, a somewhat surprising feature, since so many regard the monocotyledons as an offshoot from the dicotyledons, and base that interpretation largely upon floral morphology.

Vascular anatomy is stressed throughout the entire work, and this feature, summarizing the author's critical and extensive work along this line, is probably the most important part of the entire investigation. The carpels, as in the preceding papers, are emphasized more than the other floral parts. Some new technical terms are proposed; some old ones are discarded, the objection being made that some are not sufficiently precise. But we still retain the name cell, although as originally applied it does not cover all cells as we know them today. If precision is the object, why speak of the "fusion" of parts which were never ontogenetically separate? It is doubtful whether many of the new terms will be adopted.

On the whole, the papers and the book record the work of an industrious and competent observer. Any new point of view, with so much observation and investigation to support it, is worth careful consideration.—C. J. CHAMBERLAIN.

*The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.* By A. G. NORMAN. Oxford: Clarendon Press, 1937. Pp. ix+232. \$5.00.

Substantial gains in our knowledge of the biochemistry of the structural substances of plants have been made in recent years, particularly with reference to cellulose, the main constituent. The advances made with the other wall constituents have been much less consistent and spectacular.

These gains are given critical consideration by NORMAN, who is one of the biochemists at the Rothamsted Experimental Station. The substances considered in the monograph are cellulose, the polyuronide hemicelluloses, pentosans, hexosans, and hexopentosans, pectin, gum, mucilages, gel-forming materials, and lignin. There is a section on the metabolism of these plant cell wall constituents, and one on microbial polysaccharides. The uronic acids and pentoses receive treatment in an appendix.

It was inevitable, of course, that the author's treatment of the various constituents would be somewhat uneven, in view of the uneven progress of research on the different substances. Cellulose can be considered so much more satisfactorily than can the polyuronide hemicelluloses. Nevertheless the work covers our present knowledge accurately and admirably. It is not merely a statement of the properties and composition of the various substances; it gives an idea of the biochemical processes by which the substances arise in the plant. The author has given much attention to these structural substances in his own work, and has grasped the significance of the various trends of other research. He examines the results of current investigations critically and impartially, and

presents his conclusions so clearly and simply as to be readily understood by students of the plant sciences.

The book is particularly commended to those interested in the physiological chemistry of the life processes by which these wall substances are laid down.—C. A. SHULL.

*Potash Deficiency Symptoms.* By OSKAR ECKSTEIN, ALBERT BRUNO, and J. W. TURRENTINE. Pp. xii+235.

Attention is called to a unique publication dealing with the deficiency symptoms of many agricultural plants from lack of potash. The material has been gathered by the cooperation of the American, German, and French potash industries, and every part of the work is printed in three languages, English, German, and French. Students will be grateful for this parallel presentation of the material in several languages.

Following the introduction, the first part of the volume deals with general potash deficiency symptoms. These include the external symptoms and modifications of the inner structure of the organs, leaf, root, blossom, and fruit; secondary effects of potash deficiency, such as modified resistance to diseases, insects, nematodes, climate, etc.; potash deficiency and the market value of crops; and the pathology of potash deficiency.

The second part of the work gives details of the effects upon individual crops, such as maize and other cereals, and fruit trees. In the collection of the materials dealing with the grains, the authors had the assistance of G. N. HOFFER, and in connection with fruit tree symptoms, of G. A. COWIE.

The most attractive part of the monograph is the representation in colors of the symptoms found for individual species of crop plants. There are fifty-three of these colored plates, beautiful and instructive, and with the symptoms listed in the three languages.

This is a most commendable form of placing before the peoples of the world the facts regarding potash as a necessary plant food. Moreover, the industries have borne the expenses of making these colored plates, and supply the book at the unusually low price of \$2.25 per copy. It is distributed in the United States by B. W. Westermann & Co., 24 West 48th St., New York.—C. A. SHULL.

*General and Economic Botany.* By ERNEST ELWOOD STANFORD. New York: D. Appleton-Century Company, 1937. Pp. xxix+675.

In general, attempts to teach botany as an applied subject with little reference to its fundamentals have furnished the student no basis of understanding of plant science as such, nor has he been given more than a hodge-podge of assorted ideas concerning the problems of plant production and utilization. The present book attempts to present principles through the use of species and materials of economic importance. The illustrative material is well chosen,

much of it fresh and new, mostly direct and purposeful. The general tone of the book closely parallels the several botanical texts now popular. Similar to other comprehensive texts, academic or economic, this one will serve to supplement the inexperience and lack of direct contacts necessarily true for many of us as teachers, and will suggest further inquiry on the part of the individual student.—E. J. KRAUS.

*The Living Garden.* By E. J. SALISBURY. New York: The Macmillan Company, 1937. Pp. xi+338. Illustrated.

This is truly a different type of book, written by a botanist for those interested in gardening. Instead of the usual presentation of various rule-of-thumb suggestions, the author has attempted to present various concepts concerning plant culture from the physiological, ecological, and morphological points of view. The result is a thoroughly readable book, not equally strong in all parts, but valuable both to one interested in maintaining a garden and to any casual reader interested in plant growth and development. It might well serve as a point of departure for a series of similar books which could present to a wide range of readers the usefulness of present day botanical concepts as bases for interpretation of the practices of horticulturists, agronomists, and others dealing with living plants. The illustrations are unusually well done and adequate to the text.—E. J. KRAUS.

*The Practice of Silviculture.* By RALPH C. HAWLEY. New York: John Wiley & Sons, 1937. Pp. xiv+252.

Although intended primarily as a text on the practice of silviculture rather than a presentation of the fundamentals on which such practice must be based, ecologists generally and forest ecologists particularly cannot fail to deduct many principles on the basis of the practices suggested in this book. Methods of cutting and thinning, methods of maintaining or reestablishing stands, and methods of slash disposal are discussed in considerable detail. The diagrammatic illustrations aid in the understanding of the text.—E. J. KRAUS.

*Leguminous Forage Plants.* By D. H. ROBINSON. London: Edward Arnold & Co., 1937. Pp. vii+119. \$2.40.

Brief descriptions of the several important economic species included under eleven genera are given. These descriptions cover the seed, seedling, and more mature stages. The illustrations are fairly well drawn, adequate to the purpose for which they are intended, but lacking in critical detail. Bits of information, such as relative hardiness of some strains of these various species, feeding value of some types of hays, and the like, are added.—E. J. KRAUS.



*A List of Missouri Fungi.* By WILLIS E. MANEVAL. The University of Missouri Studies, Columbia, Missouri: The University of Missouri, 1937. Pp. 150.

This book lists alphabetically some 1191 organisms identified as to species and varieties, with about ninety others included but identified only as to genera. Included in the former group are four species of nemas, thirty-nine bacteria, and four Myxomycetes, the remainder belonging to the higher fungi. The list is based on specimens collected and deposited in herbaria and on the literature dealing with the taxonomy, distribution, and pathological activities of Missouri fungi. The hosts are named mostly according to GRAY's Manual, seventh edition, and following the list of fungi a host index and a bibliography are given.—  
J. M. BEAL.

# THE BOTANICAL GAZETTE

March 1938

## STUDIES IN THE PHYLOGENY OF THE BETULACEAE II. EXTREMES IN THE RANGE OF VARIATION OF FLORAL AND INFLORESCENCE MORPHOLOGY<sup>1</sup>

ERNST C. ABBE

(WITH ONE HUNDRED FIGURES)

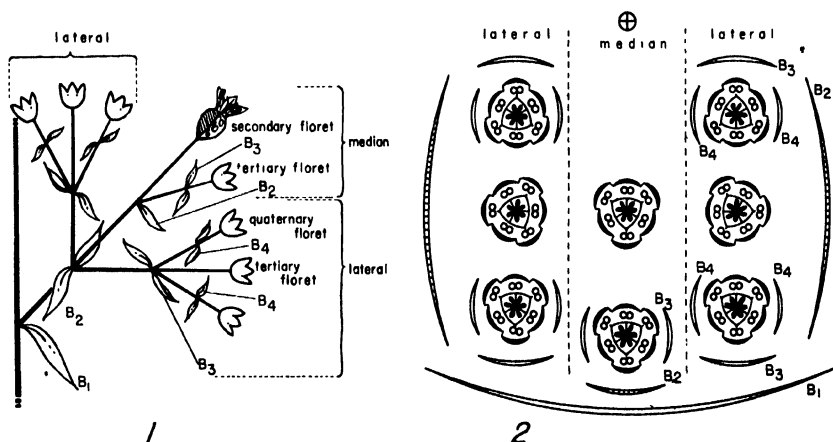
### Introduction

In the course of examining the material which forms the basis of the preceding paper of this series (I), a number of variants from the usual floral conditions were noted. Certain of these variations were described at that time, in so far as they served to clarify the more usual morphological conditions in the cymules and florets. The following report extends this survey of the floret and inflorescence morphology of the Betulaceae. Practically all of the variants, some of them frequently, occurred in normal aments. Thus they are to be considered as extremes in the possible range of normal variation rather than as teratological forms.

The variations involve either the individual florets, or the cymules, or both. They can further be classed as representing either extreme reduction or as the development of organs usually more completely suppressed.

<sup>1</sup> The observations here reported were made in part while the writer was privileged to utilize the research facilities of the Biological Laboratories, Harvard University, and of the Botany Department, Cornell University. To the authorities in charge at these institutions, the writer takes this occasion to express his thanks. The project was completed with the aid of technical assistance provided through a research grant from the Graduate School of the University of Minnesota.

The terminology used in the following descriptions is based on that established previously (1, p. 3) and is indicated diagrammatically in figures 1 and 2. In the axil of the primary bract occurs the secondary axis, bearing two sets of secondary bracts and terminating in a secondary floret. In the axils of each of the secondary bracts occur tertiary axes, each bearing a single set of tertiary bracts and terminating in tertiary florets. There is no evidence of further branching of the tertiary axis of the median system. But in the axils



FIGS. 1, 2. - Fig. 1, diagrammatic sketch of single hypothetical cymule to illustrate terminology employed. Fig. 2, floral diagram of same cymule to show inter-relationship of parts.

of the tertiary bracts of the lateral systems occur quaternary axes bearing quaternary bracts and terminating in quaternary florets.

In the following pages reference is made to the transition region from twig to ament. In some genera this region, which is immediately below the ament proper, often exhibits interesting and significant deviations from the morphological conditions in the ament. It is a region in which the internodes are progressively shorter, the leaves smaller, and the buds sometimes replaced by floral structures.

The state of union of the members of the perigon is referred to as syntepaly, a term parallel to sympetaly, synsepal, etc. Absence of tepals is referred to as atepaly, and independence of tepals as apotepaly. The methods used are essentially those which have already been described in detail (1, p. 2).

## Floral variations

### I. TRICARPELLATE PISTILS

Mention has been made of the occurrence of tricarpellate pistils in *Betula luminifera* (1, p. 55); they have also been found by the writer in *Alnus hirsuta*, *A. rubra*, *Betula glandulosa*, *B. grossa*, *B. lutea*, *B. schmidtii*, *Ostryopsis davidiana*, and *O. nobilis*. ZIMMERMANN (31) noted a floret with a tricarpellary pistil in the transition region of an ament in *Alnus glutinosa*.

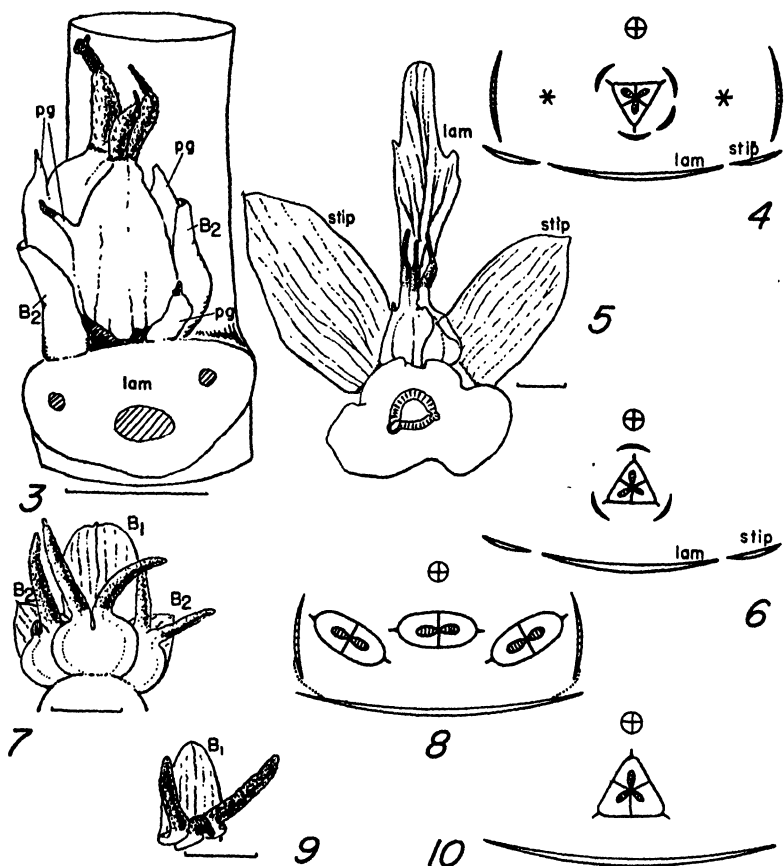
Trimerous pistils may occur in any of the three general subdivisions of the ament; namely, in the much crowded proximal and distal regions, in the main body of the ament, or in the transition region from twig to ament proper. In all the cases observed, the third carpel appeared in florets of otherwise normal aments, except in *B. luminifera* where it was associated with insect injury.

The presence of a third carpel in the ovaries of cymules from the terminal region of the ament was noted only in *B. lutea* (figs. 9, 10). The extra carpel is adaxial here, as it mostly is when there is only a single floret in the axil of a foliar structure. The effect on the position of the extra carpel in the central portion of the ament has not been determined in *Betula* and *Alnus*, because of the extremely flattened condition of the florets.

In *Ostryopsis* the tricarpellary condition was noted in both *O. nobilis* and *O. davidiana*. The same plan obtains in both species, so that the case illustrated for *O. nobilis* (figs. 11-14) may be taken as representative (for a habit sketch see 1, fig. 99). The tricarpellary floret occurs in the central portion of the characteristically loose ament. The three dorsal bundles (*dr*) of the pistil (fig. 12) arise in essentially the planes in which the bundles to the secondary bract (*B*<sub>2</sub>) and tertiary bracts (*B*<sub>3</sub>) depart from the vascular cylinder of the secondary pedicellar axis (fig. 11). Thus the extra carpel is median to the secondary bract (fig. 13). Trimery does not extend into the perigon. The orientation of the carpels in this material is superficially difficult to interpret because of the twisting and lateral flattening of the floret above the pedicel (fig. 13).

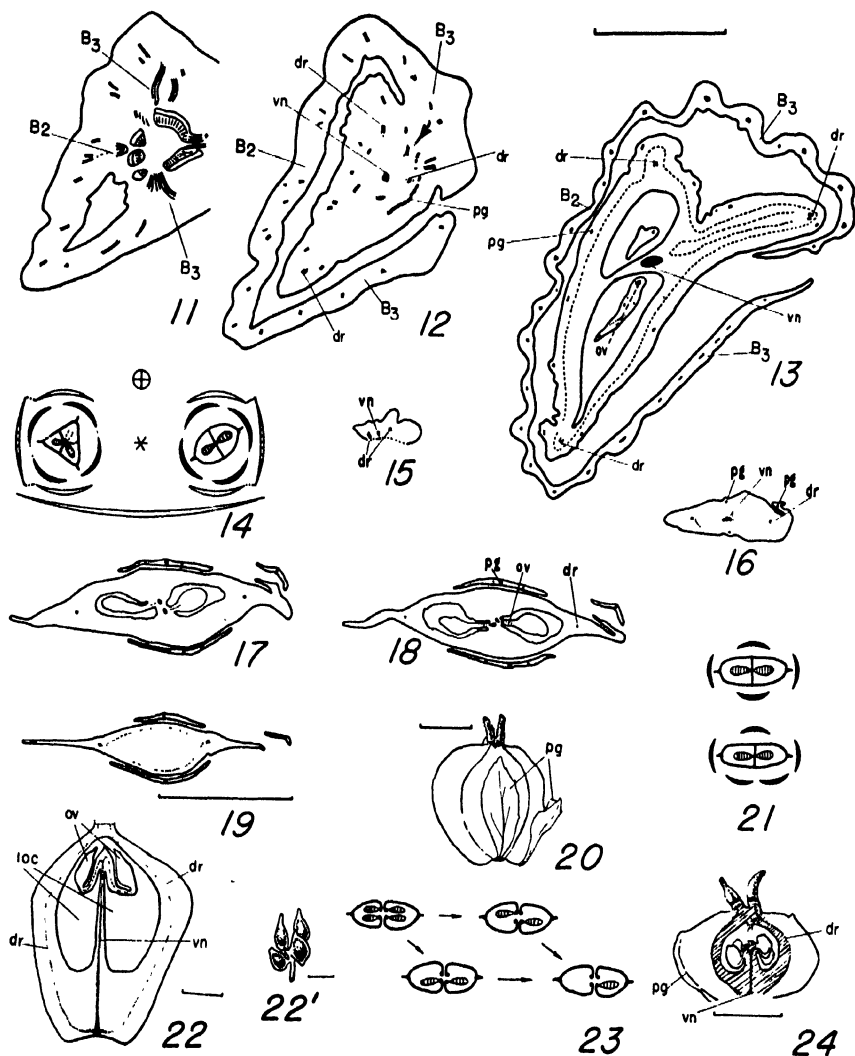
Correlated with this flattening is the lack of development of an ovule and locus in the third carpel (the adaxial carpel). The two

functional carpels are those which are represented in the usual bicarpellary ovary of this genus (compare the right and left ovaries in the floral diagram, fig. 14). This lends further support to the working hypothesis that the bicarpellary ovary of the Betulaceae originated from a tricarpellary form through the suppression of



FIGS. 3-10.\*—Habit sketches and floral diagrams respectively. Figs. 3, 4, *Betula schmidtii*, tricarpellary pistillate floret in axil of reduced foliage leaf. Figs. 5, 6, *B. grossa*, tricarpellary pistillate floret in axil of reduced foliage leaf. Figs. 7-10, *B. lutea*, pistillate; figs. 7, 8, usual type of cymule (young); figs. 9, 10, tricarpellary floret solitary in axil of bract from terminal portion of ament.

\* Scale of figures in each case indicated by a line representing 1 mm. Habit sketches and microscopic sections are all camera lucida drawings. Reconstructions of vascular systems are longitudinally exaggerated to bring out behavior of bundles to better advantage. Transverse portions of fig. 37 is to scale on a 45° projection. Abbreviations same as those used earlier (1).



FIGS. 11-24.—Figs. 11-14, *Ostryopsis nobilis*, pistillate; figs. 11-13, successive transverse sections of single tricarpeal floret with associated bracts; fig. 14, floral diagram of cymule from which sections 11-13 were made. Figs. 15-21, *Alnus rubra*, pistillate; figs. 15-19, successive transverse sections of four-ovuled floret with three tepals present; fig. 20, habit sketch of floret of type shown in figs. 15-19; fig. 21, floral diagram to show two possible types of reconstruction. Fig. 22, *A. japonica*, longitudinal section of floret. Fig. 22', *A. firma* var. *hirtella*, dissection to show the four ovules. Fig. 23, diagrams to show possible transitions from four- to one-ovuled condition in Betulaceae. Fig. 24, *Betula pumila*, longitudinal section of four-ovuled floret from base of ament.

either the adaxial or median carpel—in *Ostryopsis*, the adaxial carpel. In *Carpinus japonica*, PRANTL (19) found that three-styled pistils (presumably also three-carpelled) were of frequent occurrence. Three-parted fruits have been described for *Corylus* by MASTERS (14) and figured by WORSDELL (28), both cases being interpreted by GUILLAMIN (12) as representing a tricarpeillary pistil. In the same category occur the three-loculed ovaries of *Corylus* which SPACH (23) mentions.

Tricarpeillary pistils are oftenest found in the axils of the much reduced foliage leaves of the transition region from twig to ament. Two examples are illustrated, one of *Betula schmidtii* (figs. 3, 4) and the other of *B. grossa* (figs. 5, 6). In general the extra carpel is abaxial if secondary bracts are present (figs. 3, 4) and adaxial if secondary bracts are absent (figs. 5, 6, 9, 10), although exceptions occur.

## 2. PERIGON UNUSUALLY REDUCED OR WELL DEVELOPED

Reference was made earlier (1) to evidence for the presence of a rudimentary perigon in the pistillate florets of many of the Betuleae. As was pointed out at that time (p. 60), four lines of evidence support this concept: the developmental, the anatomical, the presence of glands at the base of the styler column, and the rare occurrence of foliose tepals.

The best examples of foliose tepals in the pistillate florets of the Betuleae were found in a single collection of *Alnus rubra* (fig. 20). A large proportion of the florets had two or three tepals of the type shown in figure 20. The tepals are usually fused with the ovary wall only near the base. When they occur against the flat side of the ovary their shape is lanceolate to oblanceolate, the margins sparingly toothed. The teeth evidently were originally gland-tipped, although in most cases the glands had fallen off in drying (this being herbarium material). The tepals which occur opposite the margins of the flattened ovaries are often dwarfed and crumpled, as if distorted by restrictions of space within the ament. A summary of the various conditions observed is represented diagrammatically in figure 21, the perigon being either tetramerous or pentamerous. The presence of both tetramerous and pentamerous florets in the same species and even in the same cymule is by no means infrequent in the staminate

aments of *Alnus*, and it is therefore not surprising to find such tendencies in the pistillate aments. Serial sections of one of these pistillate florets (figs. 15-19) show the distinct nature of the vascular supply to the tepals. The material was mature at the time of collection, so that a well defined, heavily staining abscission layer had formed at the base of the floret. As a result the precise details of the origin of the supply to the tepals were not determined, although it is clear that the bundles to the tepals do not become independent until after the ventral and the dorsal bundles of the carpels depart from the pedicellar vascular supply.

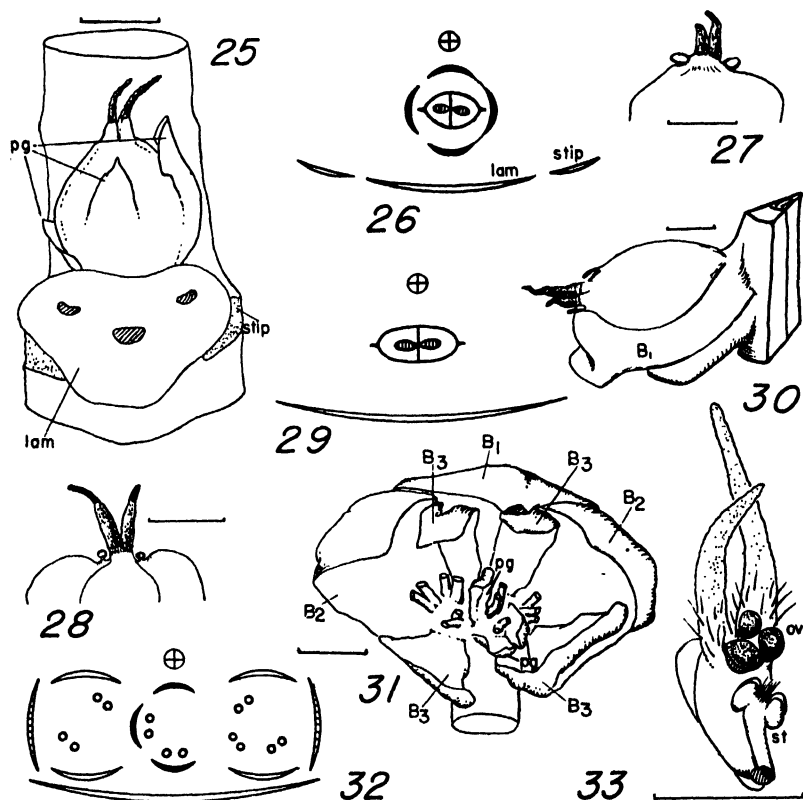
ZIMMERMANN reports (30) and figures (31) pistillate florets with tetramerous perigons in each of which the tepals are arranged as they are in the staminate florets. These florets were found in the region intermediate between the staminate and pistillate portions of a bisexual ament of *Alnus glutinosa*.

Tepals similar to those occurring in the material of *A. rubra* just described are to be found in florets occurring in the axils of reduced foliage leaves in the transition region in *Betula medwediewii* (pg, figs. 25, 26), *B. schmidtii* (pg, figs. 3, 4), *B. pumila*, and *B. lutea*. Figure 25, illustrating the condition in *B. medwediewii*, strikingly shows the variable behavior of the tepals, from complete freedom on the one side to fusion for half their length on the other side of the floret. This suggests that recrudescence of the foliose tepal in pistillate florets of the Betuleae does not necessarily provide reliable evidence as to whether the ovary was formerly superior or inferior.

The transition from the rarely foliose tepal in the pistillate florets of the Betuleae to the distinctive glands near the base of the style is easily understood, since similar glands occur on the margins of the tepals in the florets of *Alnus rubra* previously described, as well as regularly on the margins of the tepals in the staminate florets of many species of *Alnus* and *Betula*. So usual is the reduction from foliose tepal to gland within the same ament, or even within the same cymule in staminate florets of *Betula*, that the locus occupied by a conspicuous gland in one floret is clearly recognizable as that occupied by a foliose tepal in another nearby floret. Thus in *B. maximowicziana*, a staminate cymule was chosen in which the adaxial tepal (1, fig. 219, x) of the secondary floret is represented



only by a characteristic gland. Had another cymule been chosen for illustration from the same ament, tepal  $\alpha$  might well have been foliose, since many of that type were observed in that species. More



FIGS. 25-33.—Figs. 25, 26, *Betula medwediewii*, pistillate floret with tepals in axil of reduced foliage leaf from base of ament, habit sketch and floral diagram respectively. Fig. 27, *Alnus tenuifolia*, habit sketch of upper part of floret to show glands. Fig. 28, same, of *Betula maximowicziana*. Figs. 29, 30, *B. globispica*, tricarpeal floret with glands, solitary in axil of primary bract, from base of ament, floral diagram and habit sketch respectively. Figs. 31, 32, *Corylus vilmorinii*, teratological staminate cymule with tertiary bracts and tepals present, habit sketch and floral diagram respectively. Fig. 33, *Betula luminifera*, teratological bisexual floret with extruded ovules.

reduced species, such as *B. pendula* (1, fig. 198) or *B. lenta* (1, fig. 181), have a larger proportion of the tepals of the individual florets represented by glands only. Of considerable interest from this point of view is PAYER'S (18) report of four tepal primordia for each

staminate floret in *Betula* "*alba*," although not all of these primordia develop into tepals.

In pistillate florets these glands, which represent the apex of a tepal otherwise fused to the ovary wall, vary in number, shape, and constancy of occurrence from species to species, and sometimes may possibly be of taxonomic value. Rarely there are four, as sometimes in *Alnus subcordata* (1, fig. 41); more commonly there are two of varying form in the plane of the dorsal bundles, as in *A. sibirica*, *A. incana*, *A. maritima*, *A. spaethii*, *A. tenuifolia* (fig. 27), *A. nepalensis*, *Betula globispica* (fig. 30), *B. coerulea-grandis* (1, fig. 98'), *B. utilis* var. *pratensis*, and *B. maximowicziana* (fig. 28). Vestigial bundles, whose course suggests that they are remnants of a perigon supply, occur in *Alnus incana*, *A. subcordata*, and *Betula pumila* (fig. 24).

Foliose tepals, when they occur in the pistillate florets, are usually well vascularized. On the other hand, in the tepals of the staminate florets of the Betuleae, a vascular supply may be absent or be represented only by the terminal fragment of a trace disconnected from the main vascular system of the cymule. A corresponding behavior may characterize the much reduced adaxial tertiary bracts sometimes present in the pistillate cymules of *Alnus*. The occasional lack of continuity of the vascular system of a much reduced foliose organ with that of the rest of the cymule suggests an arrested stage in the normal development of most foliar organs—a condition associated, perhaps, with the small *absolute* size attained by these organs when they stop growth. In fact, they are so small at times that they survive without a vascular system. The objections raised by ARBER (2) concerning the phylogenetic significance of vestigial traces may perhaps be met on the basis of an analysis of the material concerned in terms of absolute size. It is physiologically unlikely that relatively large organs would persist without a vascular system. Yet relatively small organs, such as the rudimentary glumes of the Gramineae cited by ARBER and the vestigial tepals and bracts of the Betulaceae, are conceivably capable of survival without a vascular system, and of course upon their complete loss cannot leave vestigial bundles behind them. Let the absolute size of the flowering structure as a whole be greater and the organs involved be correspondingly larger, then non-functional but existent organs may be expected in many cases

still to have vascular systems, and since space restrictions are less, to leave vestiges of these vascular systems to survive their disappearance.

In the staminate cymules of the Coryleae, whose florets are usually atepalous, it is only in *Corylus* that tepals have been found in the writer's material. These occur with relative frequency near the base of the ament in otherwise normal *C. americana* (1, figs. 253, 254) and *C. maxima* (1, figs. 255, 256), the tepals represented being either the adaxial or the abaxial ones of the secondary floret. As pointed out previously (1), the key to the interpretation of these as tepals lies in their vascular supply, which is intimately associated with that of the corresponding stamen in the same fashion as that to be found in homologous structures in the staminate florets of *Alnus* and *Betula*. WEISS (26) found two tepals to be associated with a dimerous secondary staminate floret in a bisexual cymule from an androgynous ament in "hazel."

A situation which is associated with a disturbance of the aments sometimes occurs in staminate cymules of *Corylus vilmorinii*. These cymules are notable because of the full development of the perigon of the secondary floret and the occasional presence of tepals in the tertiary florets, as well as for the presence of a full complement of tertiary bracts. In the example illustrated (figs. 31, 32) the three tepals of the secondary floret were associated with two stamens (four half-stamens). In other cymules as many as five tepals and three stamens were present in the secondary floret, while sometimes the tertiary florets had a single tepal each and three stamens. In spite of the fact that the material of *C. vilmorinii* is teratological, the phenomena observed fit so well into the basic pattern of the betulaceous floret and cymule that it must be considered as a significant expression of the genic potentialities of the group.

The absence of tepals usually present has been noted in *Alnus glutinosa* in the staminate florets in several instances, especially in florets from the transition region.

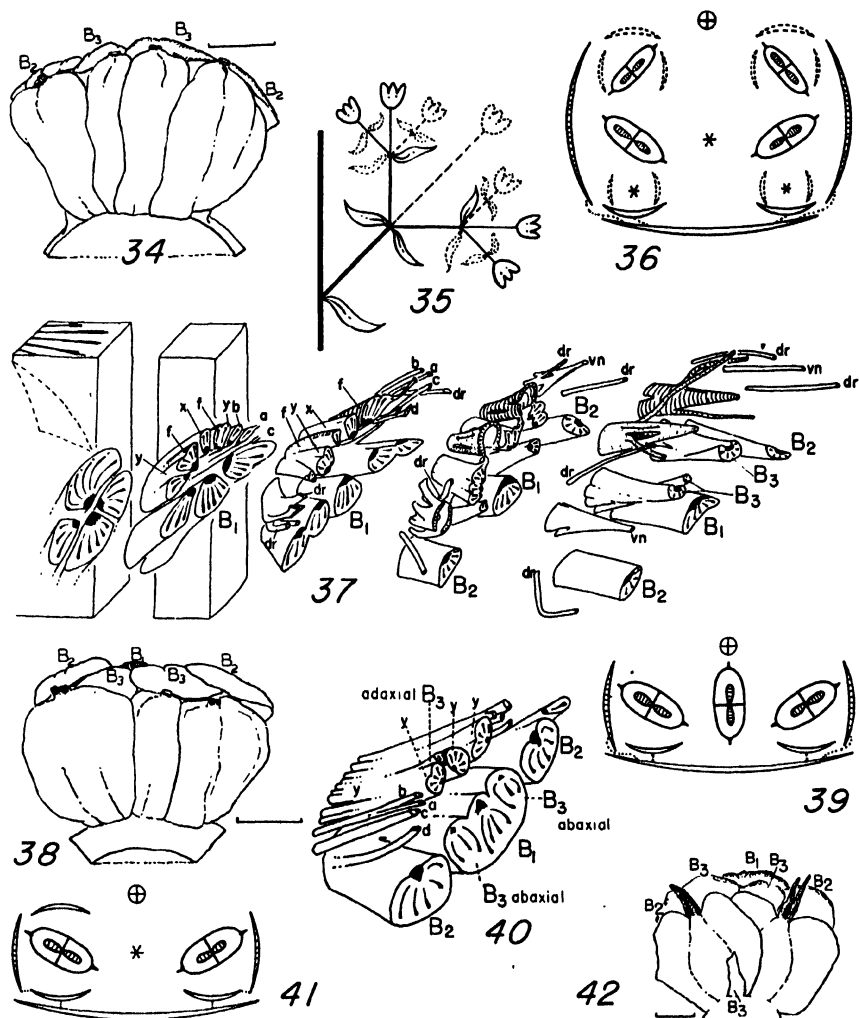
### 3. VARIATIONS IN OVULE NUMBER

The number of ovules usually ascribed to the ovary in the Betulaceae is two, as shown for *Alnus japonica* (fig. 22). The two loculi

(*loc*), which are poorly defined, usually become continuous through disappearance of the septum above the insertion of the ovules. The disappearance of the septum is associated with the failure of the ventral bundle (*vn*) to extend beyond the insertion of the ovules (*ov*). Sometimes the union of the two loculi occurs below the insertion of the ovules, so that parietal placentation is newly attained. Basically the placentation is axile, since the ventral bundle exists as a single compound structure in most species of the family. Furthermore, tricarpeillary ovaries in the family never have parietal placentation, but are consistently axile.

Occasionally in *Alnus firma* var. *hirtella*, *A. rubra*, and *Betula pumila*, more than two ovules occurred. Four ovules were present rather frequently in *Alnus firma* var. *hirtella*, there being one ovule for each margin of the two carpels (fig. 22'). A similar situation was noted by WOLPERT (28) in *A. alnobetula*. In the material of *A. rubra* already described, in connection with the presence of a foliose perigon, four or even five ovules are frequently present, the vascularization of a four-ovuled floret being shown in figures 15-19. In figure 17 is shown the departure of traces to ovules on diagonally opposite margins of the two adjacent carpels, while in figure 18 the ovules of the other two margins are being vascularized. The traces to the lower pair of ovules become distinct at a relatively low level in the ovary (fig. 16) and continue parallel to the ventral bundle (*vn*) for an appreciable distance upward while it splits preparatory to supplying the upper pair of ovules. There is no vestigial vascular material extending beyond the insertion of the upper pair of ovules (fig. 19). PAUCHET (17) reports three ovules, two in one carpel and one in the other, as sometimes present in the ovary of *Corylus avellana*, not an unexpected finding since BAILLON (6) had noted that the anlagen of four ovules are to be found in the pistils of *Corylus* although but rarely did he find all four completely developed. In *Betula pendula* there may sometimes be two ovules to a carpel, according to STREICHER (24).

The same general condition as described for *A. rubra* also occurs in the floret of *Betula pumila*, shown split longitudinally in figure 24. This is rather uncommon in *Betula*, the floret illustrated being from the transition region at the base of the ament.



FIGS. 34-42.—Figs. 34-39, *Alnus subcordata*, pistillate; figs. 34-37, four-flowered cymule from central portion of ament, habit, diagram of inflorescence, floral diagram, and reconstruction of vascular system respectively; figs. 38, 39, three-flowered cymule, habit and floral diagram respectively. Fig. 40, *A. lanata*, pistillate cymule, reconstruction of vascular system, adaxial tertiary bract present (cf. fig. 41). Figs. 41, 42, *A. crispa* var. *mollis*, pistillate cymule with adaxial tertiary bract present, floral diagram and habit respectively.

The series of schematic diagrams shown in figure 23 represent the possible transitions from the four-ovuled ovary to a potential one-ovuled ovary in the Betulaceae. The four-ovuled diagram may also be considered to represent the rarely observed five-ovuled condition. The fifth ovule is superposed on any one of those already present. Intermediate between the four-ovuled and the two-ovuled state is the presence of three ovules, a condition which was observed in a number of species. The three-ovuled condition could then give rise to either of the two-ovuled conditions shown, by the loss of one or the other of the ovules on the margins of the same carpel. No case has been observed of one of the carpels becoming completely sterile and the other bearing two ovules. Either of the two-ovuled conditions may occur within the same cymule (as shown in fig. 44, and 1, fig. 150). In most of the genera of the Betulaceae both of the ovules usually present develop somewhat beyond the fertilization stage, but frequently only one of these matures. This tendency reaches its extreme in *Corylus*, where two-ovuled fruits are rare. The trend in the family appears to be definitely toward the one-ovuled condition, representing the ultimate in a reduction series from a multi-ovulate pistil. Should a one-ovuled type of pistil ultimately become the rule in the family, the development of an equitant ovule (comparable with that of the Juglandaceae) is conceivable, although this is highly unlikely since it would necessitate the return of an-anatropous ovule to an orthotropous condition.

#### 4. VARIATIONS IN NUMBER OF STAMENS

Comment has already been made (1) on the variability in the number of stamens in the florets of *Alnus*, *Betula*, *Carpinus*, and *Ostrya*. The androecium in *Ostryopsis* and *Corylus* is usually variable within much narrower limits than in the other genera mentioned. The presence of an exceptional increase in the number of stamens in *Corylus* florets has already been described in discussing the presence of tepals in these florets. SCHULZ (22) reports the occurrence of unusual numbers of stamens in *Corylus avellana*, although he did not find tepals in his material. His observations may be interpreted as being examples of (a) reduction to monomery in each of the three

florets of the cymule, (b) monomery of the tertiary florets and trimery of the secondary floret, and (c) monomery of the tertiary florets and tetramery of the secondary floret. BAILLON (8, p. 225, footnote 3) mentions such variations in more general terms for *Corylus*, while WYDLER (29) early recognized the variability of stamen number in *Alnus*.

Monomerous androecia in the florets from the transition region of aments of *Alnus glutinosa* (31) and monomerous or dimerous androecia in *Ostrya carpinifolia* and *O. virginica* (15) have been noted.

In the remarkably reduced cymule of *Alnus* s. *Cremastogyne* there may sometimes be a total of five stamens instead of four distributed among the three florets (21), indicating either trimery of the secondary floret or dimery of one of the tertiary florets.

#### 5. BISEXUAL FLORETS AND SEX REVERSAL

Bisexual florets in the Betulaceae are rare in the experience of the writer. One case, observed in *Betula luminifera*, is teratological since it is associated with insect injury. Here, in the same ament which produced the tricarpellary ovary already referred to, there also occurred a bisexual floret (fig. 33). A clearly recognizable stamen (*st*) is fused by its filament with the ovary wall. In this same floret the ovules (*ov*) are extruded through an enlargement of the interstylar canal, adding to the bizarre appearance of the floret.

Bisexual florets were also found in an apparently normal pistillate ament of *B. lenta*. The lowermost three or four cymules of one collection have individual florets which exhibit various conditions. The filaments of the stamens in some are long and fused, for most of their length, with the wall of the ovary with which they are associated—the anthers remaining free, however. In others the filaments are short, the anthers being practically sessile at the base of the ovary.

The third case in which bisexual flowers were noted was in the transition region of a pistillate ament of *B. pumila*. Here it is not clear whether the stamens represent the tertiary florets and the ovary a secondary floret, or whether all are associated together as members of the secondary floret. In several other specimens from the same region there appear to be staminodes opposite some of the

tepals. A similar condition occurred in *B. globispica*. The latter were the only cases where there were structures of any sort in the cycle between perigon and gynoeceium, but the morphological identity of these is by no means clear.

In the many staminate cymules studied, a careful watch was maintained for external evidences of vestigial ovaries. These were not found, nor is there vascular tissue in the staminate florets which suggests a vestigial ovary supply. Other workers, however, have found hermaphroditic flowers in various species. BAIL (4) reports them in *Alnus incana* in the transition zone of a bisexual ament. In the staminate flower there was a rudimentary ovary between two of the stamens. In otherwise normal *A. glutinosa* he observed a similar condition, as well as in *Corylus avellana*. WORSDELL (28) found a single stamen in the female flower of *Carpinus betulus*. SCHULZ (22) observed a number of hermaphroditic florets. In the basal region of pistillate aments of *Alnus glutinosa* he found florets which, in their most complete state, had perigon and androeceium similar to those of the staminate florets and a gynoeceium like that of the pistillate florets (the orientation of the latter being either median or transverse to the primary bract). He also found hermaphroditic flowers at the base of staminate aments. In *Betula* "alba," SCHULZ found florets at the base of staminate and pistillate aments, which, like those of *Alnus glutinosa*, combined the features of the usual staminate and pistillate florets. Interestingly enough, the orientation of the pistil here was also sometimes median to the primary bract.

In these florets the perigon and androeceium were sometimes tetramerous. SCHULZ also found as a very rare occurrence in *Corylus avellana* the presence of hermaphroditic flowers at the base of a pistillate ament. Here the secondary floret of the cymule, which alone was present, had a tiny perigon, four stamens, and a bicarpellary ovary. VON MERCKLIN (15) figures and describes a bicarpellary ovary accompanied by two stamens inserted below the ovary in *Ostrya virginiana*. WEISS (26) found bisexual florets in the region between staminate and pistillate portions of androgynous aments of "hazel," as well as cymules in which the secondary floret is staminate and the tertiary ones are pistillate. Associated with anomalous branching of the staminate catkins of *Corylus avellana*, MÜLLER-STOLL (16) found



pistillate branches rarely developed, in one of which a pistil had two stamens accompanying it.

Two examples of sex reversal were observed by the writer, both in the transition region of a pistillate ament of *Betula lenta*. In the axils of two of the much reduced foliage leaves characteristic of this region there were stamens associated with tepals, forming a staminate floret instead of the pistillate floret which usually occurs in such positions (fig. 54).

WEHRLI (25) reports for *C. avellana* the complete replacement of the four stamens in otherwise normal staminate cymules, with an equal number (rarely three or five) of long red stigmas. This he considers evidence in support of the theory that the male and female elements of the flower (in general) are identical.

HÖSTERMANN (13) has found pistillate flower buds in the transition region of staminate aments in *Corylus*. He quotes PENZIG as reporting this phenomenon to be a regular occurrence in *C. maxima*. BAIL (4) found a staminate floret in the transition region of a pistillate ament of *Alnus glutinosa*.

ZIMMERMANN (31) has noted numerous cases of the replacement of anthers by carpels in staminate florets of *A. glutinosa*. He has also noted in the same species florets which have a tetramerous perigon, dimerous androecium, and dimerous gynoecium centrally located.

VON MERCKLIN (15) reported the replacement of one or of both anthers by one or two carpels in both *Ostrya carpinifolia* and *O. virginiana*. These occurred in the terminal region of a considerable number of staminate aments. The florets in this region were reduced to monomery, as is so often the case in the terminal portions of aments.

### Inflorescence variations

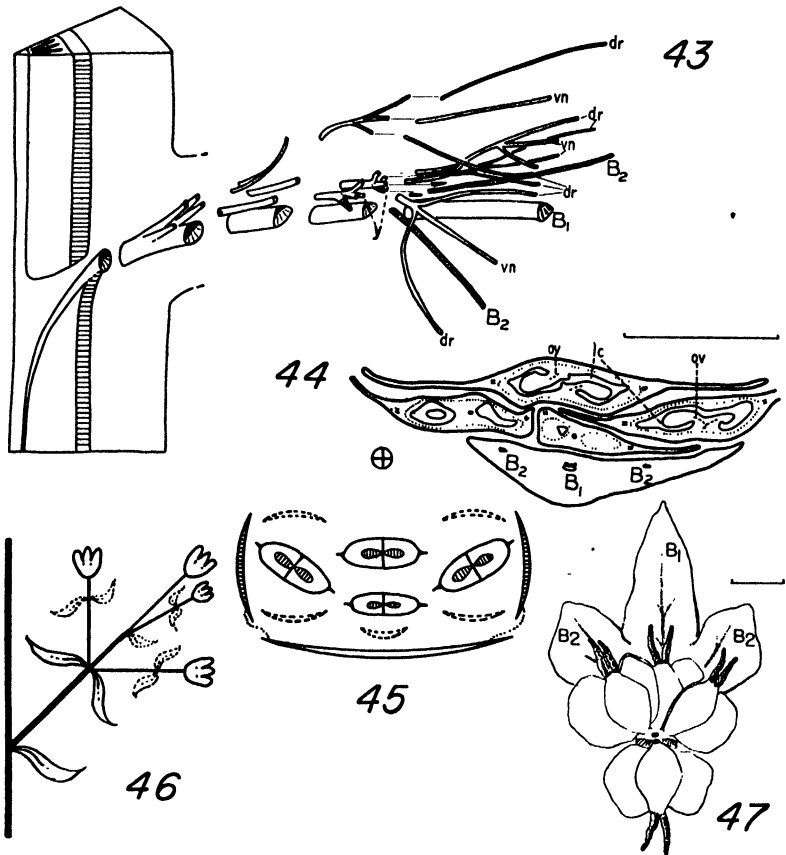
#### 1. PRESENCE OF BRACTS USUALLY ABSENT

Bracts which are usually absent occur most frequently in the pistillate cymules of *Alnus*. Here one or the other of the adaxial tertiary bracts, more or less reduced, characterizes one or more collections of *A. jorullensis*, *A. crispa* var. *mollis* (fig. 42), and *A. lanta* (1, fig. 64). As many as half of the cymules in the ament may have an adaxial tertiary bract, especially in *A. crispa* var. *mollis*.

The most extreme development of the adaxial tertiary bract was found in *A. lanata* (1, fig. 64). Associated with its relatively large size is the clarity of the relationship of its vascular supply to that of the rest of the cymule. In figure 40 is shown that part of a three-dimensional reconstruction of the vascular system of the cymule mentioned which indicates the origin of the vascular supply to the adaxial tertiary bract (*adaxial B<sub>3</sub>*). It should be noted that the bundle to the adaxial tertiary bract arises from a gap between the bundles  $\gamma$  and  $\gamma$ , which represent in part the vascular material of the tertiary axis. A strictly comparable situation characterizes the departure of the bundle to the adaxial tertiary bract in the pistillate cymules of *A. jorullensis*, when one or the other of these bracts is present. In *A. crispa* var. *mollis*, where the vestigial adaxial tertiary bracts are smaller, when present (fig. 42), the vascular system of the bract may be continuous with that of the rest of the cymule, or else may be entirely discontinuous if the adaxial tertiary bract is very small (1, figs. 25-34). In the various species which occasionally possess adaxial tertiary bracts, there seems to be a definite correlation between the absolute size of the vestigial bract and the extent to which it is vascularized, the structure still being recognizable after direct continuity of its vascular system with that of the rest of the cymule has been lost. This condition apparently represents an arrested state of the ordinary course of events in the development of the vascular system of a foliar organ. The further significance of the presence of the adaxial tertiary bract will be considered in connection with the presence of quaternary florets in *A. subcordata*.

There was found in the Arnold Arboretum a specimen of *Carpinus caroliniana* with an unusual development of small subsidiary pistillate aments in the axils of the variously reduced foliage leaves, immediately below the bases of the usual aments. A great variety of conditions was noted in the individual cymules. The subsidiary aments have from one to three or four nodes. The proximal cymule of the subsidiary aments is usually sterile and is represented by two large secondary bracts more or less fused with the small primary bract. In the second or third cymule there are from one to three florets, the most complete development of bracts and florets observed being shown in figure 50. In this cymule there is an additional

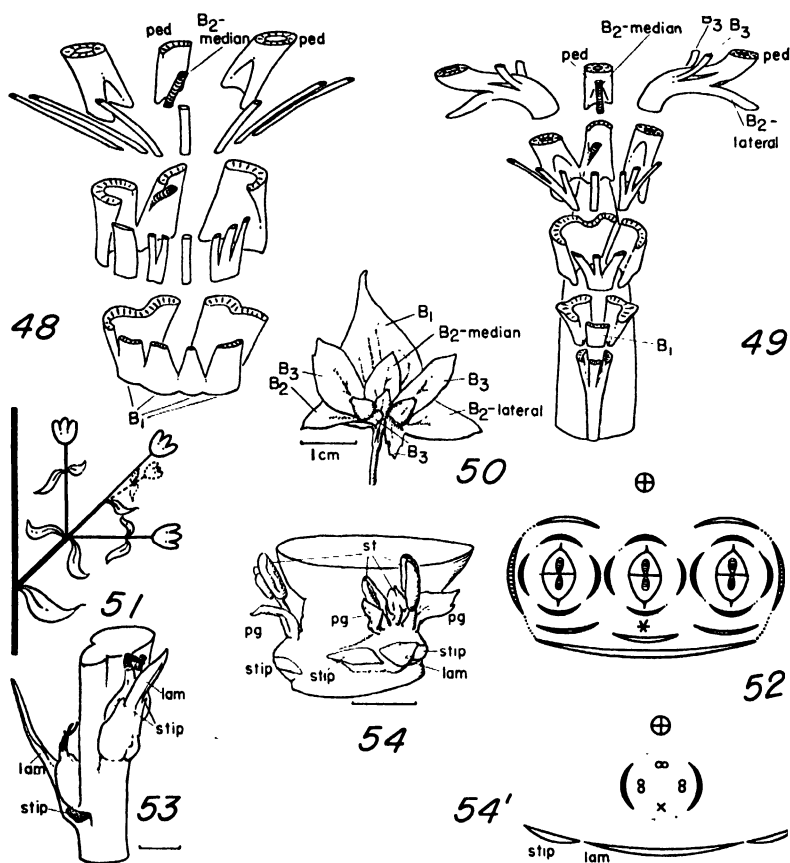
bract in the median plane which is designated as the median secondary bract (fig. 50, *B<sub>1</sub>-median*). The vascularization of this particular cymule is shown in figure 48, where it will be noted that the trace



FIGS. 43-47.—*Betula papyrifera* var. *occidentalis*, four-flowered pistillate cymule; fig. 43, reconstruction of vascular system; fig. 44, cross section of cymule near middle of florets; fig. 45, floral diagram of cymule; fig. 46, diagram of inflorescence; fig. 47, habit sketch of cymule, uppermost floret turned down.

to the median secondary bract arises from the rather poorly defined vascular cylinder of the secondary axis. This suggests that the bract involved is at the second node on the secondary axis (fig. 51). Striking confirmatory evidence for this view comes from

the presence of a floret in *Betula*, which has a similar relationship to the vascular system of the secondary axis and will be discussed on a



FIGS. 48-54'.—Figs. 48-52, *Carpinus caroliniana*, pistillate cymule; fig. 48, reconstruction of vascular system of cymule shown in fig. 50; fig. 49, reconstruction combining characteristics of vascular systems of several extreme types of cymule; fig. 50, habit sketch of cymule whose anatomy is shown in fig. 48; fig. 51, inflorescence diagram of fig. 50; fig. 52, floral diagram of fig. 50. Fig. 53, *Betula schmidtii*, basal region of pistillate ament to show transition from reduced leaf with its stipules to their fusion. Figs. 54, 54', *B. lenta*, staminate florets in axils of reduced foliage leaves at base of pistillate ament and floral diagram of anterior floret respectively.

subsequent page. ZIMMERMANN (31) describes in *Alnus glutinosa* a pistillate cymule which appears to have the median abaxial tertiary and quaternary bracts present, although this interpretation of his

figures and descriptions is open to question (see his numbers 19 and 20, 1918).

In staminate material of *Carpinus*, there has already been noted (1, figs. 239, 241 and discussion) the occurrence of free secondary bracts in the staminate cymules at the proximal end of the ament in *C. japonica*. ZIMMERMANN (31) noted this same occurrence in a species of *Carpinus* (*C. betulus?*). This evidence, in addition to the fact that the vascular system of the secondary bracts is present in the apparently simple "primary" bracts in most material of *Carpinus*, suggests that this primary bract is in reality a compound structure representing the lateral fusion of the primary bract with the secondary bracts.

The teratological staminate aments of *Corylus vilmorinii*, already referred to in the discussion of tepals, also have occasional cymules in which both the adaxial and abaxial tertiary bracts are well developed (figs. 31, 32). This is a significant case because the tertiary bracts are regularly absent in normal staminate material. In contrast, the tertiary bracts are fully developed in the pistillate cymules, forming the "involucral husk." All four of the tertiary bracts are not always present in the teratological staminate cymules, although at least two are generally represented.

The freedom of one or the other of the tertiary bracts from the secondary bracts is noted in pistillate *Carpinus betulus* var. *heterophylla* by ČELAKOVSKÝ (10), while he also notes variation toward the opposite extreme from the average; namely, nearly complete fusion of a tertiary with the secondary bract. PRANTL (19) observed the freedom of the adaxial tertiary bract in *C. japonica*, and indicates that this is the structure variously referred to by earlier taxonomists in their descriptions.

## 2. ABSENCE OF BRACTS USUALLY PRESENT

In the staminate and pistillate aments of *Alnus* and *Betula* and in the staminate aments of *Corylus*, the number of bracts usually present in the central portion of the ament is often reduced in the cymules at the extreme proximal and distal regions of the aments. This is illustrated by the absence of the secondary bracts in the single-flowered cymule from the distal portion of an ament of

*Betula lutea* shown in figure 9, as contrasted with the presence of the secondary bracts in a cymule from the central portion of the same ament (fig. 7). It is this type of phenomenon occurring normally in every ament under stress of space restrictions which makes more easily understandable the phylogenetic reduction from the type of cymule found in *B. alnoides* var. *pyrifolia* (1, figs. 97, 98) to the simpler type of *B. luminifera*. There is a similar transition from the pistillate cymules of the other birches of the Nanae group (1, figs. 80, 82) to those of *B. michauxii* (1, fig. 84), or from the staminate cymules of the alders where secondary and some tertiary bracts are present to those of *A. lanata* (1, figs. 177, 178), in which only the primary bract is evident externally. Intermediate stages occur in *A. firma* var. *hirtella* (1, figs. 156, 157) and in *A. maritima*, where the tertiary bracts are in a state of impending loss or else gone entirely as in some specimens of *A. crispa* var. *mollis* (1, fig. 158) and of *A. alnobetula* (27). This is given as a general condition in *Alnus*, subgen. *Alnaster*, series *Firmae*, by CALLIER (9) in his revision; his data for this characterization he credits to SCHNEIDER.

### 3. PRESENCE OF FLORETS USUALLY ABSENT

The occurrence of the secondary floret in pistillate cymules, where ordinarily only the two lateral tertiary florets are present, was found in *Alnus crispa*, *A. jorullensis*, *A. nitida*, *A. subcordata* (figs. 35, 36), *A. incana* (22), *A. glutinosa* (22, 31), and *Carpinus caroliniana* (fig. 50). The description and interpretation of the condition in *Alnus crispa* var. *elongata* (20) have been taken up in great detail elsewhere (1, pp. 6-9, figs. 8-19). The three-flowered cymules occurred on a plant, otherwise normal, whose pistillate aments are more or less elongate at the base. In this region occur a number of three-flowered cymules whose vascular anatomy is extremely advantageous for study and interpretation, since they are less telescoped than those of the normal ament. The evidence clearly establishes the fact that the central floret of the cymule is the secondary floret. It also provides a standard of comparison for the more reduced cymules of the central portion of the same aments as well as for the three-flowered cymules in other species.

Three-flowered cymules in the other species of *Alnus* mentioned

occurred in the central portion of the aments, where the secondary floret varies from nearly the usual size (*A. subcordata*, fig. 38) to a mere vestige (some of the specimens of *A. jorullensis* and *A. nitida*). In all of the three-flowered *Alnus* cymules which were sectioned, the vascular system was essentially similar to that described for *A. crispa* except for minor modifications induced by their dorsiventral compression. When the secondary floret is extremely reduced in size there is a corresponding reduction in the extent of its vascular supply. In some cases a vascular behavior simulating that of the pistillate cymules of *Betula* occurred. It is significant that WOLPERT (27) found rather frequently in early stages of the development of pistillate cymules in *Alnus* that a primordium representing the secondary floret is present, but later usually aborts, very rarely persisting to the mature stages.

The case of the presence of a secondary floret in *Carpinus caroliniana* (fig. 50) has already been described in some detail in connection with the presence of the median secondary bract. In other cymules than the one illustrated, a secondary floret is also often present. ČELAKOVSKÝ (10), discussing variation in the pistillate cymules of *Carpinus betulus* var. *heterophylla*, described several cases of the presence of the secondary floret in addition to the tertiary florets, as well as alone. In *C. betulus* three-flowered pistillate cymules were noted by SCHULZ (22).

A diagrammatic summary based on serial sections of three-flowered pistillate cymules of *C. caroliniana* studied by the writer is shown in the reconstruction (fig. 49). They are characterized in most cases by the development of an independent pedicellar cylinder (continuation of the secondary axis) which supplies the secondary floret. In one case, however, the secondary floret was supplied by only a single branch from the vascular cylinder of one of the tertiary florets, a well defined vascular cylinder not being formed. The vascular system of these three-flowered cymules of *Carpinus* is strikingly similar in its essentials to that of the three-flowered cymules of *Alnus crispa* (1, fig. 19).

Pistillate cymules of *Corylus* may also be three-flowered, owing to the presence of the secondary floret, according to SCHULZ's observations on *C. avellana* (22) and WEISS's observations on "hazel" (26).

The latter describes as an interesting variant a cymule in which the secondary floret was staminate and the tertiary ones pistillate.

An interesting case occurred in *Betula papyrifera* var. *occidentalis* where there are ordinarily three florets in each pistillate cymule. In several aments from one collection the greater number of cymules in the proximal portion of the ament have four florets (fig. 47; the uppermost of the two median florets is turned back to permit all four florets to be seen). The vascularization of this same cymule is shown in figure 43, from which it will be noted that the usual condition in this group (cf. 1, fig. 67) is augmented by a vascular supply to the second of the median florets. The bundles  $y$  and  $y$  continue on to vascularize the second median floret after contributing to the first median floret, and finally end in stubs, as do their homologues in *B. lenta* (1, fig. 67).

The vascular supply to the second of the median florets in the four-flowered cymules indicates clearly that this floret is attached to the continuation of the vascular system of the secondary axis of the cymule. This floret (fig. 46) is at a higher node on the abaxial side of the secondary axis in a position which corresponds to that occupied by the median secondary bract described in *Carpinus caroliniana* (fig. 51).

Another type of four-flowered cymule was encountered in the aments of *Alnus subcordata*, which have already been referred to as occasionally producing three-flowered cymules. The two additional florets lie side by side between the usual lateral secondary florets (fig. 34). In the reconstruction of the vascular system of this cymule (fig. 37) the vascular supply to the two unusual florets is indicated by stippling for the one and by cross-hatching for the other. The vascular system of the cymule departs from the plan characteristic for *Alnus* only in the presence of the bundles  $f$  and  $f$ , which develop into the pedicellar supplies of the two extra florets. Significantly, these pedicellar supplies originate from a gap left by the branching off of bundle  $y$  on either side away from  $x$  (which represents a fusion of the two central  $y$  and  $y$  bundles). A comparison of this mode of departure of the vascular supply to the two extra florets with the mode of departure of the bundle to the adaxial tertiary bract in *A. lanata* (adaxial  $B_3$ , fig. 40) suffices to demonstrate the close rela-



tionship between them. Here again in one species the foliar organ is present in the same location as that from which the supply to the floret arises in another species, and again it is possible to conclude that both originate from the same node. Thus there is present a quaternary set of florets in the axils of the adaxial tertiary bracts, where they might well be expected.

#### 4. ABSENCE OF FLORETS USUALLY PRESENT

Mention has already been made of the consistent tendency in the subfamily Betuleae for the number of bracts and of florets per cymule to be reduced in the distal, and to a less extent, in the proximal regions of the ament. In the pistillate cymules of *Betula* the number of florets may be reduced from three to two (simulating pistillate *Alnus*), as noted by the writer in *B. nana* and *B. papyrifera* var. *occidentalis* and by WOLPERT (27) for *Betula* sp. Or there may be a loss of the tertiary florets and the persistence of the secondary, the average condition in *B. michauxii*, occurring as a reductional extreme in the terminal portions of the aments in many species of *Betula*. The latter condition has also been noted by SCHULZ (22) in hermaphroditic cymules of *Betula* "*alba*." Similarly in *Alnus* the number of florets may be reduced to one in each cymule. SCHULZ notes this in certain pistillate cymules of *A. glutinosa* and *A. incana*. On the other hand, in the staminate cymules of *Alnus*, the secondary floret may be absent, as the writer has observed in *A. tenuifolia* (1, pp. 31, 32) and in *A. jorullensis* (cf. fig. 67), SCHULZ in hermaphroditic cymules of *A. glutinosa*, ZIMMERMANN (31) in staminate cymules from bisexual aments of *A. glutinosa*, and WOLPERT (27) in *A. alnobetula*.

In the Coryleae the writer has found reduction of the cymule to one floret only in *Carpinus caroliniana*, in the stunted aments at the bases of certain pistillate aments. An unusual development is described by WEISS (26) for "hazel," which had three-flowered pistillate cymules. Occasionally he found the three-flowered cymule reduced to the one-flowered state, the one flower representing the secondary floret, which in the average cymule is completely absent. SCHULZ (22) found staminate cymules in *Corylus avellana* in which only the tertiary florets (each monomerous) had developed, while

BAILLON (8) notes the occurrence in *Corylus* of only the secondary floret (also monomerous). In pistillate *Corylus* there is often not only a reduction of the individual cymule to one functional fruit (representing a tertiary floret) but also a functional reduction of the entire ament to this single fruit. While almost all of the cases of reduction observed result in a bilaterally symmetrical condition, the not infrequent behavior of pistillate *Corylus* stands apart because of the asymmetrical disposition of the fruit. This is, of course, associated with an earlier loss of the secondary floret of each cymule, followed by a further failure of one of the two tertiary florets to function.

#### 5. FLORETS IN AXILS OF FOLIAGE LEAVES

Many examples of pistillate florets in the axils of reduced foliage leaves were found, notably in the transition region in *Betula*. The following were the source of one or more examples: *B. glandulosa*, *B. grossa*, *B. lenta*, *B. lutea*, *B. maximowicziana*, *B. michauxii*, *B. nana*, *B. nigra*, *B. papyrifera* var. *occidentalis*, *B. pumila*, *B. schmidtii*, and *Alnus hirsuta*. All of these were found in the transition region and showed in common the tendency for the presence of only a single floret in the axil of the reduced foliage leaf (figs. 3, 5, 25, 53), the floret in each case replacing the axillary bud. Only very rarely are secondary bracts present (fig. 3). The number of tepals associated with the floret varies from one to the next (figs. 3, 25), while the pistils are sometimes tricarpellate (figs. 3, 5, 53) or four-ovuled (fig. 24).

The leaves in whose axils these florets occur are generally restricted to the two or three nearest the ament proper and are much reduced, the leaf blade (*lam*, fig. 5) being of nearly the same size as the stipules (*stip*). Sometimes immediately below the ament the reduced stipules are fused laterally with the blade (*lam*, upper node, fig. 53), forming a single foliose organ equivalent to the primary bract.

In staminate material the transition region is but seldom productive of unusual cases, the one case observed in *Carpinus japonica* having been already figured (1, fig. 241). Here a single cymule composed of a small group of stamens occurs in the axil of a much

reduced foliage leaf. The only example of a solitary staminate floret was observed in the axil of a reduced foliage leaf in the case of sex reversal in *Betula lenta* (fig. 54), where a small group of stamens associated with tepals occurred at two different nearby nodes.

A rare occurrence is that described by GERTZ (11) for *Alnus glutinosa*. He found in the lower part of an elongated pistillate catkin the individual florets replaced by miniature pistillate aments, some of which even had a "transition region." This is a noteworthy occurrence, indicating that even in the much reduced *Alnus* pistillate flower there still exists the potentiality for development into a more extensive leafy axis (in this case fertile).

ZIMMERMANN (31) describes in *Alnus glutinosa* numerous cases of solitary florets which are staminate, pistillate, and hermaphroditic in the axils of reduced foliage leaves in the transition region. He also observed solitary staminate and pistillate florets in a similar position in *Betula*.

#### 6. MIXED INFLORESCENCES

A number of partly pistillate and partly staminate aments have been reported. They are especially interesting in the transition zone from staminate to pistillate cymules, since hermaphroditic florets sometimes occur there (noted previously under the heading Bisexual florets and sex reversal). HÖSTERMANN (13) noted a pistillate inflorescence of *Corylus maxima* which was staminate in the upper portion. BAIL found similar situations in *Alnus incana* (4), *Betula "alba"* and *B. humilis* (3); SCHULZ (22) reports bisexual aments in *Alnus incana* and *A. glutinosa*; BAILEY (5) for *Alnus "serrulata"*; and BAILLON (7) for *Alnus* sp.

Specimens of *Alnus glutinosa* in which several pistillate aments became staminate in their upper portions and one case of the reverse condition have been noted by ZIMMERMANN (30, 31). In the transition from one sex to the other he found numerous bisexual flowers and other aberrations, many of which have been mentioned here under the appropriate headings.

Pistillate inflorescences occurred in the center of groups of bunched staminate aments in *Corylus avellana*, according to MÜLLER-STOLL (16).

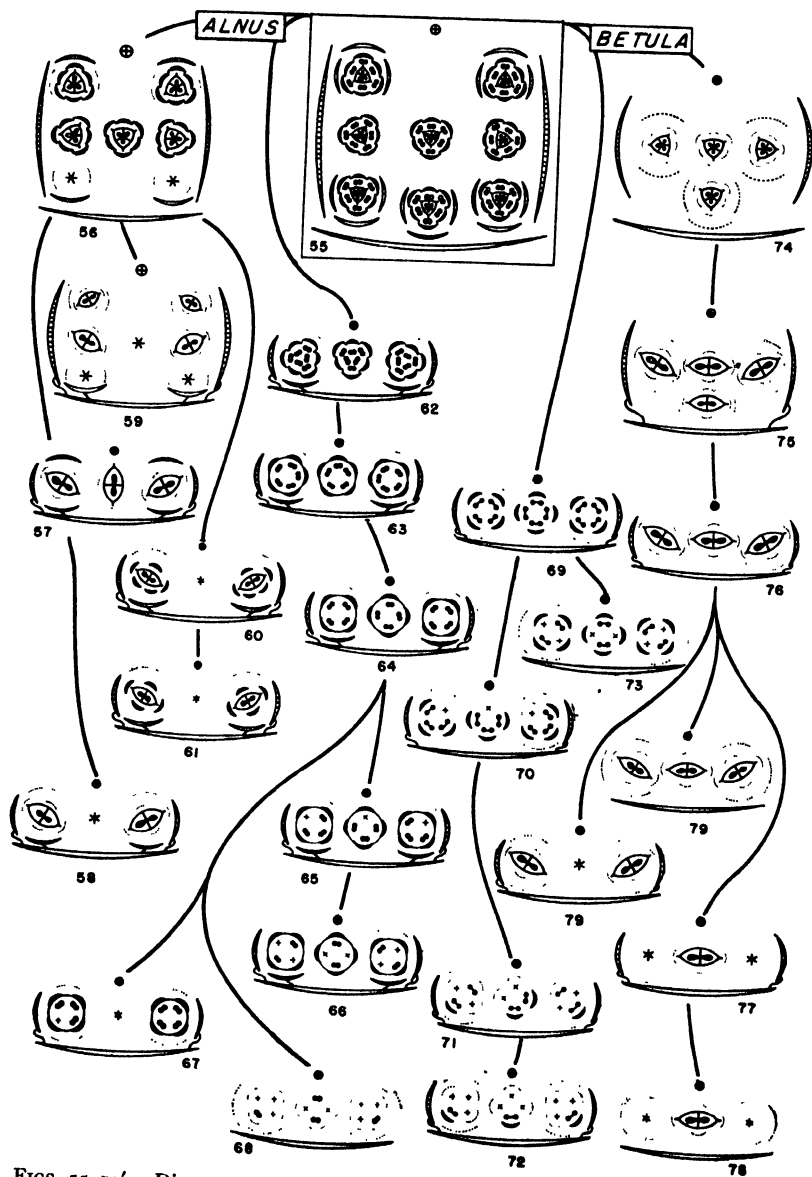
### Range of floral variation in the Betulaceae

It is now possible to summarize the range of variation in the florets and inflorescences of the Betulaceae. This is shown diagrammatically in figures 55-100, with reference throughout to a synthetic floral diagram (figs. 55, 80) which is a summary of the average conditions plus the more complex variations. Dotted lines are used for the organs which have not been found, but which serve to complete the cymose plan. In each genus the range of variation is shown as progressing from the more complex (presumably the more primitive) conditions through various stages of simplification (reduction). It is not implied that the species which form the basis for any of the diagrams are progenitors of other species represented by other diagrams. The species cited merely form the basis for an estimate of the morphological changes taking place in the family. A phylogenetic classification will be suggested at a later date, when the evidence from fields other than floral structure has been presented. Morphologically the family is of great interest because of the wide range of variation, the florets sometimes varying from hexamery to monomery and from syntepaly to atepaly within the same genus, and the cymules ranging from a four-flowered to a one-flowered state with corresponding reduction in number of bracts.

It is to be understood throughout this discussion that statements concerning conditions which are described as characteristic of certain subdivisions of the family are based on the observations of the writer. While the observations of others have been referred to and an attempt made to interpret them in the main body of this paper, the writer does not include them in this section, since the data provided are often insufficient to serve as a basis for complete floral diagrams.

#### BETULEAE (FIGS. 55-79)

**ALNUS, PISTILLATE CYMULES (FIGS. 56-61).**—The more complex pistillate conditions are summarized in figure 56. Here is embodied the tricarpellary condition of the pistils from the transition region, the presence of a perigon, and the presence of adaxial tertiary bracts and quaternary florets, all of which are extremes found in one or another of the species of the genus. The perigon is shown as hex-



FIGS. 55-79'.—Diagrammatic summary of range of variation in florets and cymules of Betuleae.

amerous because the conditions observed in *A. rubra* may well be derived from such an arrangement, and it is found on occasion in the staminate florets of other species. Syntepaly is indicated because the presence of glands on the upper part of the ovaries suggests an even more complete fusion of the perigon with the pistil than that which is associated with syntepaly in the Coryleae. The average type of two-flowered pistillate cymule is shown in figure 58. This could be derived morphologically either from the four-flowered condition shown in figure 59 (based on *A. subcordata*) by the loss of the quaternary florets, or from figure 57 (based on *A. crispa* var. *elongata* and *A. subcordata*) by the loss of the adaxial tertiary bracts and of the secondary floret, or from figure 61 (based on *A. rubra*) by the loss of the tepals. While there is a considerable range of variation in the pistillate cymules of *Alnus*, the great majority of average cymules for each species studied falls in with the diagram shown in figure 58.

ALNUS, STAMINATE CYMULES (FIGS. 62-68).—There is great variation in the average condition of the individual florets and practically no variation in the number of florets per cymule from one section to the next. Thus, in the s. *Alnobetula*, florets tend to be hexamerous or pentamerous (figs. 62, 63), with occasional further reduction to tetramery or trimery, while one or both of the abaxial tertiary bracts may frequently be absent (not shown in the diagrams). In the s. *Gymnothyrsus*, tetramery (fig. 64) or trimery (fig. 65) is frequent, with occasional dimery (fig. 66), all with the abaxial tertiary bracts usually present. In the s. *Clethropsis* the individual florets range from pentamery (fig. 63) through trimery (fig. 65), the cymules usually having the abaxial tertiary bracts present. In a phylogenetic scheme the members of this section would follow a separate line because of the marked abbreviation of the cymules in length, a characteristic which does not lend itself to representation in the floral diagrams. Finally, in the interesting s. *Cremastogyne* (fig. 68), not only are the tertiary bracts absent as they so often are in the s. *Alnobetula*, but the androecium is monomerous or dimerous as in the more reduced members of s. *Gymnothyrsus*, the cymule is short like that of the species of s. *Clethropsis*, and, unlike any of the other members of the genus, the secondary bracts are absent and the

florets are atepalous. In each of these groups, except the last, the average conditions are flanked by extremes which parallel in one or more ways the average condition in the other groups. One type of variant (fig. 67, based on *A. tenuifolia* and *A. jorullensis*) which occasionally develops is of interest because of the absence of the central floret, thus resulting in a situation like that of the pistillate cymule.

BETULA, PISTILLATE CYMULES (FIGS. 74-79').—Figure 74 represents a synthesis of the more complex variations observed in this genus and embodies, in addition to the average structure, the presence of the abaxial median tertiary floret and of trimerous gynoecia. The four-flowered cymule (fig. 75, based on *B. papyrifera* var. *occidentalis*) is of peculiar interest, since it expands the median portion of the synthetic diagram for the family (fig. 55) into a several-flowered group, thus supplementing the contribution from the four-flowered cymules in *Alnus* (fig. 59). By the loss of the abaxial median tertiary floret the average condition in the ss. Costatae, ss. Albae, and part of the ss. Nanae is reached. By the further loss of the lateral tertiary florets (fig. 77, based on *B. nana*), followed by the loss of the secondary bracts (fig. 78, based on *B. michauxii*), the extremes in range of average conditions in the ss. Nanae are realized. In the ss. Acuminatae the average condition varies from that of figure 76 (as in *B. luminifera*) to the incipient and finally complete absence of the secondary bracts (fig. 79', based on *B. alnoides* var. *pyrifolia*). A variant, characterized by the absence of the secondary floret, is of interest because it simulates the average condition in *Alnus* pistillate cymules (fig. 79, based on *B. nana* and *B. papyrifera* var. *occidentalis*).

BETULA, STAMINATE CYMULES (FIGS. 69-73).—There is a continuous series from the apotepalous tetramerous to a monomerous condition in both perigon and the androecium. The tetramerous state (fig. 69, based on hybrid [?] *B. maximowicziana*) is unusual and gives way to the trimerous (fig. 70) and dimerous (fig. 71) florets characteristic of the members of the ss. Costatae (with trimery not infrequent) and the ss. Albae (with dimery of the androecium and monomery of the perigon more frequent than in the ss. Costatae). In the ss. Nanae dimery of androecium and monomery of perigon

finally go over into monomery of both (fig. 72, based on *B. pumila* etc.). Divergent (except for *B. maximowicziana*) from these sections is the ss. *Acuminatae* (fig. 73) in the loss of the secondary bracts (present in all of the other ss.) and the tendency of the androecium to be reduced in number of parts before the perigon suffers such loss. In this latter respect the ss. *Acuminatae* shows an interesting parallelism to the occurrences in the staminate florets of *Alnus*.

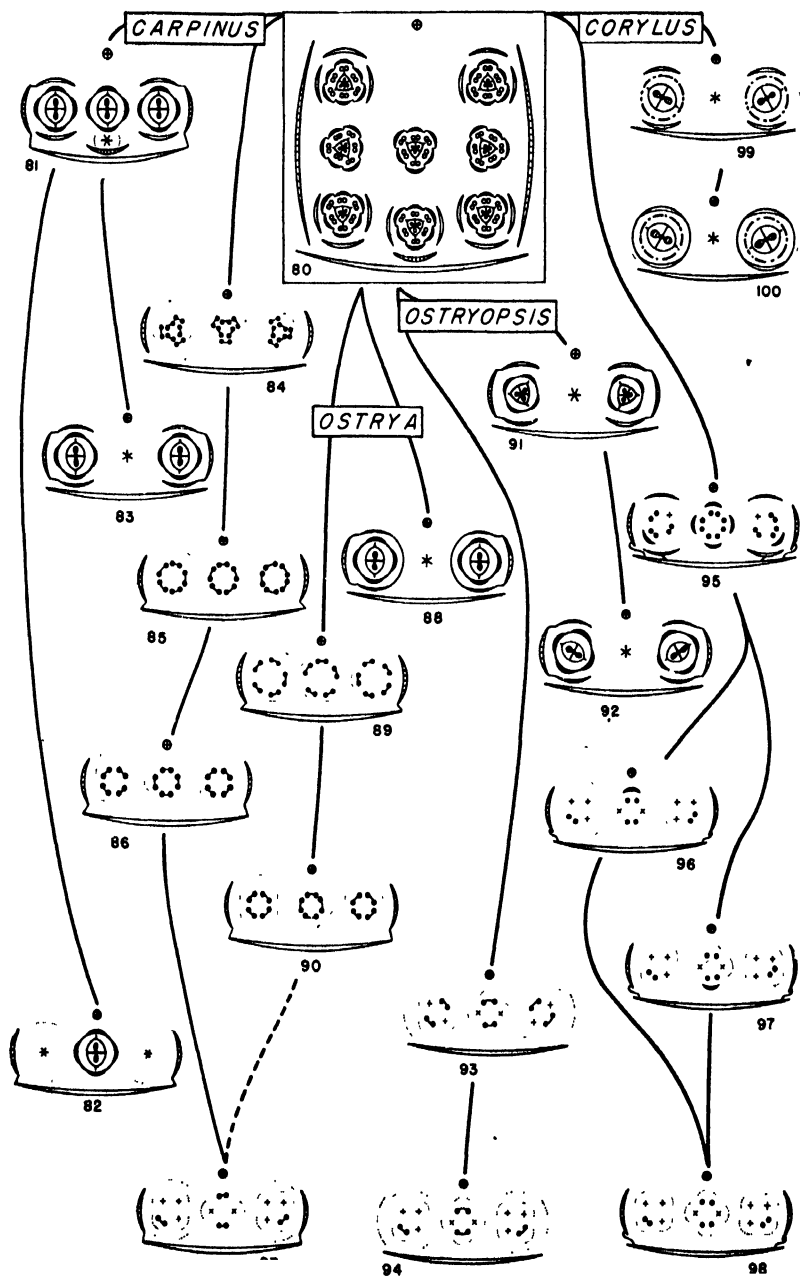
#### CORYLEAE (FIGS. 80-100)

CARPINUS, PISTILLATE CYMULES (FIGS. 81-83).—The average condition in both sections of the genus is that shown in figure 83. Somewhat less reduced is the situation in which the secondary floret is present (fig. 81, based on *C. caroliniana*) and rarely accompanied by the abaxial median secondary bract. The latter circumstance is of especial interest in reconstructing a hypothetical ancestral form (figs. 80, 55), since it is in the axil of a bract in this location that the fourth floret occurred in one extreme case in pistillate *Betula* (fig. 75). A rare extreme in reduction is due to the loss of the tertiary florets and of the tertiary bracts (fig. 82, based on *C. caroliniana*). The latter is of interest not only because it represents a radically different organization from that of the average cymules in the genus (fig. 83), but also because it parallels an extreme of reduction found in the pistillate cymules of *Betula* (fig. 77).

CARPINUS, STAMINATE CYMULES (FIGS. 84-87).—The individual florets range from a hexamerous (fig. 84) to a dimerous or monomeric (fig. 87) state, depending on their location in the ament, and are notable in contrast with most of the *Betuleae* because of the absence of the perigon. It is, however, of interest to find that the same extreme of reduction is reached here as in some species of the *Betuleae*, although it is not so fixed that it becomes a species character. While the secondary bracts are mostly so completely fused laterally with the primary bract as to be indistinguishable externally, occasionally they are free, as indicated in figure 84 (based on *C. japonica*).

OSTRYA, PISTILLATE CYMULES (FIG. 88).—Very little variation was observed, the florets and cymules consistently assuming the condition shown in figure 88.





FIGS. 80-100.—Diagrammatic summary of range of variation in florets and cymules of Coryleae.

OSTRYA, STAMINATE CYMULES (FIGS. 89, 90).—The range of variation closely parallels that in the staminate cymules of *Carpinus*, with the exception that the largest number of parts per floret is apparently less than it is in *Carpinus*. No examples of free secondary bracts have come to the writer's attention, although there is internal vascular evidence of their existence.

OSTRYOPSIS, PISTILLATE CYMULES (FIGS. 91, 92).—Occasional three-carpelled ovaries occur (fig. 91, based on *O. nobilis*), providing a transition to the usual two-carpelled ovary (fig. 92).

OSTRYOPSIS, STAMINATE CYMULES (FIGS. 93, 94).—The extent of reduction of the staminate florets is remarkable because of the strong resemblance between the pistillate florets of the genus and those of *Carpinus* and *Ostrya*. Yet there is in the average staminate florets of the genus a state of reduction to dimery (fig. 93) or monomery (fig. 94) which corresponds in extent only to extreme conditions in *Carpinus* and *Ostrya*. In this respect the floral structures correspond more closely to those of *Corylus*, although *Corylus* still retains the secondary bracts which are not evident (even internally) in *Ostryopsis* (cf. also fig. 68).

CORYLUS, PISTILLATE CYMULES (FIGS. 99, 100).—In both groups of species, those with the bracts fused laterally to form the "husk" (fig. 100) and those in which the bracts are free (fig. 99), the structure is uniform. The extraordinary absence of secondary bracts while both of the tertiary bracts are still present is also a very constant feature. The variation in the basic morphology of the florets is slight.

CORYLUS, STAMINATE CYMULES (FIGS. 95-98).—The average condition is that shown in figure 98, a combination of monomery and dimery in the various florets. In some cases perigon segments occurred (figs. 96, 97) which form a transition to the usual completely atepalous florets. The least reduced state (fig. 95, based on *C. vil-morinii*) is not only supplied with perigon in the florets but the usually absent tertiary bracts are also present in their entirety.

### Discussion and conclusions

Although a consideration of the phylogenetic ramifications within the family is best left until other lines of evidence have been brought

to bear upon it, it is still desirable to attempt the synthesis of an ancestral type of floret and cymule. The present effort will be restricted to the reconstruction of a hypothetical form which combines the least modified characteristics observed in the various members of the family.

The question arises as to the validity of including in such a reconstruction the deviations from the average which are described in the main body of this paper. The objection which will be raised most frequently is that these deviations are *abnormal*. Normality is a difficult state to define objectively. Moreover, it is easily confused with the more restricted concept which may be referred to as *average*. In the previous paper of this series (1) it was the average condition which was stressed; namely, the condition which exists in most of the cymules in the major portion of the ament. But in these same aments, mostly at the base or apex, there occur cymules whose structure is either more simple or rarely more complex than that of the rest of the ament. These less usual forms must be considered as normal, since they uniformly occur in association with average cymules. They are slightly different expressions of gene complexes which presumably are the same in every vegetative cell of the plant. The difference in expression of similar gene complexes can best be attributed to slightly different spatial relationships of the parts involved, and perhaps to slight physiological differences. Thus the identical gene complexes in two adjacent florets of the cymule may be associated in one floret with the presence of only two tepals while in its neighbor there may be three tepals. Or, in another case, a floret in the apical region of the ament may have but one tepal; a floret in the central region, three; and a floret at the base, four tepals—apparently correlated with difference in position and probably also with physiological factors. Yet in all of them the genic complement is almost certainly alike throughout. All of these states are normal, but that which occurs most frequently, and thus is characteristic of the species, is to be regarded as the average.

Of the two extremes of variation from the average in a species, that of reduction is most easily recognized as foreshadowing the average in closely related species. In blocking out phylogenetic trends, at least these reductional extremes may be considered sig-

nificant. Good circumstantial evidence supporting this assumption is to be found in the pistillate cymules of *Betula*. Composing the average pistillate cymule of *Betula* ss. *Albae*, there are three bracts and three florets (fig. 76); yet in the same ament in which such cymules predominate, there are more reduced cymules in the crowded apical region of the ament. These latter cymules may sometimes be two-flowered (fig. 79) or in other cases one-flowered (fig. 77). In the ss. *Nanae*, with its smaller aments, this same range of variation is also frequently found. Often the entire ament will be composed of uniflorous cymules (as for instance in *B. nana*), but with each floret there is ordinarily the usual set of three bracts. In the terminal region of these aments, however, there may be only a single bract associated with the single floret (fig. 78). This latter condition, extreme in *B. nana*, is the average for *B. michauxii*. Briefly, the reductional extreme occurring in a region of crowding in one species is the average condition in the next more reduced species.

Not only are the extremes of reduction significant, but the opposite extreme often reflects the average in a less reduced species. But in the least reduced species this involves the presence of one or more organs which are absent in the average state of that least reduced species. Specifically, into this category in the Betulaceae come the florets which occur in the axils of the adaxial lateral tertiary bracts, the abaxial median secondary bract and the floret in its axil, the third carpel in the pistil, and the bisexual type of floret sometimes provided with a perigon. When these structures occur, they are in practically all cases associated with average conditions to the same extent as the opposite extreme (reduction). There is no more reason to assume that there are different genic factors involved in the development of these more complex cymules than in the development of more reduced ones. Furthermore, the less reduced conditions fit in at one end of the average range of variation as smoothly as do the more reduced states on the opposite end (figs. 55-58, 55-78, etc.). These more complex cymules may represent simply the result of interaction between more favorable external influences and the same internal hereditary factors that are expressed to a less degree in the reduced cymules of the same species under less favorable circumstances. It would seem that the less reduced conditions

are as significant in their phylogenetic implications in the Betulaceae as are the more reduced conditions.

The use to which these extremes of non-reduction may be put is clear. From each of the genera come fragments of evidence pointing toward a less modified type of floret and cymule. By fitting the bits of evidence together, a hypothetical ancestral type may be reconstructed. As shown in figures 55 and 80, this is a less reduced cymule than the average for the family. It should be noted that the hexamerous state of the individual florets is considered by the writer to be merely a stage in a reduction series from some remote, less reduced ancestral form which had a larger number of tepals in the perigon. The trimerous state shown represents the arrangement naturally taken by the tepals when only six are present and distributed on the basis of well known phyllotactic laws. There is no implication of monocotyledonous affinities in the trimerous state indicated; it merely represents the uppermost point in the wide range of variation characteristic of the family.

The different genera contribute various items to the reconstruction of a hypothetical ancestral form. From pistillate *Alnus* there are added to the basic three-flowered cyme the two adaxial quaternary florets and the adaxial tertiary bracts; from pistillate *Betula*, the abaxial median tertiary floret; from pistillate *Carpinus*, the abaxial median secondary bract. The remaining florets are added because the bracts subtending them are present, and because it is assumed that the cymule is, as its name suggests, basically cymose. That the latter may be a gratuitous assumption will be taken up in more detail elsewhere.

The individual floret of the reconstruction derives its two-cycled, trimerous perigon and androecium, as well as its syntepaly, primarily from staminate *Alnus*. The insertion of the stamens is in doubt, but was probably at the base of the free part of the tepal. The validity of indicating the floret as hermaphroditic is suggested by the cases of this sort observed in the transition from staminate to pistillate portions of androgynous aments, etc. The tricarpellary pistil is based on such pistils in *Alnus*, *Betula*, and *Ostryopsis*. The inferior tricarpellary ovary is based on *Ostryopsis*. The placentation is indicated as axile because this is the condition in the well developed

tricarpellary pistils which have been observed. The ovary probably had three completely distinct loculi. The number of ovules in each carpel was at least three (*cf.* figs. 1 and 2).

In attempting to allocate a position to the Betulaceae among the groups to which it is allied, these groups might be expected to have inflorescences and florets which can be derived from a plan closely similar to that worked out here for the Betulaceae. The Fagaceae come to mind, but further consideration of this problem must await a broader basis for comparison.

### Summary

1. A number of the cymules and florets whose complexity is greater or less than the average in the Betulaceae are described. The more complex cymules include the presence of: the secondary median floret in pistillate *Alnus* and *Carpinus*; adaxial quaternary florets in pistillate *Alnus* and adaxial tertiary bracts in pistillate *Alnus* and staminate *Corylus*; the abaxial median secondary floret in pistillate *Betula*; and the abaxial median secondary bract in pistillate *Carpinus*. The more complex florets include the presence of: tricarpellary pistils in *Alnus*, *Betula*, and *Ostryopsis*; a hexamerous perigon and androecium in staminate *Alnus*; and three ovules per carpel in *Alnus*. A few cases of hermaphroditism were observed.

2. The more reduced conditions in the cymule include the loss of: the secondary floret in staminate *Alnus* and pistillate *Betula*, the tertiary florets in pistillate *Betula* and pistillate *Carpinus*. The more reduced conditions in the floret include the reduction of the androecium to monomery and the perigon to atepaly.

3. The less reduced conditions are summarized in a synthetic ancestral form. The characteristics of the cymule of this ancestral form would be as follows: a series of racemosely arranged cymose (?) inflorescences, each composed of a median triflorous group and two lateral triflorous groups, with an appropriate complement of bracts; the individual hermaphroditic flowers equipped with hexamerous perigon (probably syntepalous), hexamerous androecium, the gynoecium inferior, tricarpellary, with axile placentation and three or more anatropous ovules.

It is a pleasure to acknowledge the help derived from discussing this problem with Professor F. K. BUTTERS of the University of Minnesota, Professor A. J. EAMES of Cornell University, and Professor R. H. WETMORE of Harvard University.

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# A PHOTOKYMOGRAPH FOR THE ANALYSIS OF THE AVENA TEST

C. L. SCHNEIDER AND F. W. WENT

(WITH FOURTEEN FIGURES)

## I. Introduction

In the *Avena* technique, when an agar block containing auxin is placed unilaterally on the cut surface of a decapitated coleoptile, it will bend because of the increased growth of the side under the agar block. This technique, the *Avena* test, is extensively used for the quantitative determination of auxin. This paper describes results obtained with a photokymograph which was designed to record the procedure of these curvatures automatically.

It is known that, under prescribed conditions, these curvatures are proportional to the concentration of auxin in the blocks. This proportionality has been described by WENT (15), VAN DER WEIJ (13), VAN OVERBEEK (8), and SKOOG (11). Nonconformity, however, was found by NIELSEN (7) and SÖDING (12). Since the work of VAN DER WEIJ, no systematic analysis of the conditions most favorable for the test has been carried out, and the accepted procedure is partly a matter of chance. Thus a more detailed analysis of these curvatures has a practical value, besides its theoretical significance.

The first attempts to express the auxin curvature as a function of time were made by DU BUY and NUERNBERGK (1) and by DOLK (2<sup>1</sup>), in both cases by measuring the pictures of curving plants on film strips taken by a lapse time movie camera. The measurement and calculation require an excessive amount of work and time, however, so that this method has fallen into disuse.

Both VAN OVERBEEK (10) and SKOOG (11) give data on the procedure of the auxin curvatures under different conditions; but the time intervals between successive observations are long (one hour or more) and the measurement (with a protractor) does not allow for high precision, as required if finer details of the curvatures have to

<sup>1</sup> See figure 51 in WENT and THIMANN (17).

be analyzed. Besides, VAN OVERBEEK (10) has shown that the light necessary for photographing the plants influences the subsequent stages of curvature.

## II. Method

The most desirable method of recording the procedure of the auxin curvatures ought to give a continuous permanent recording without

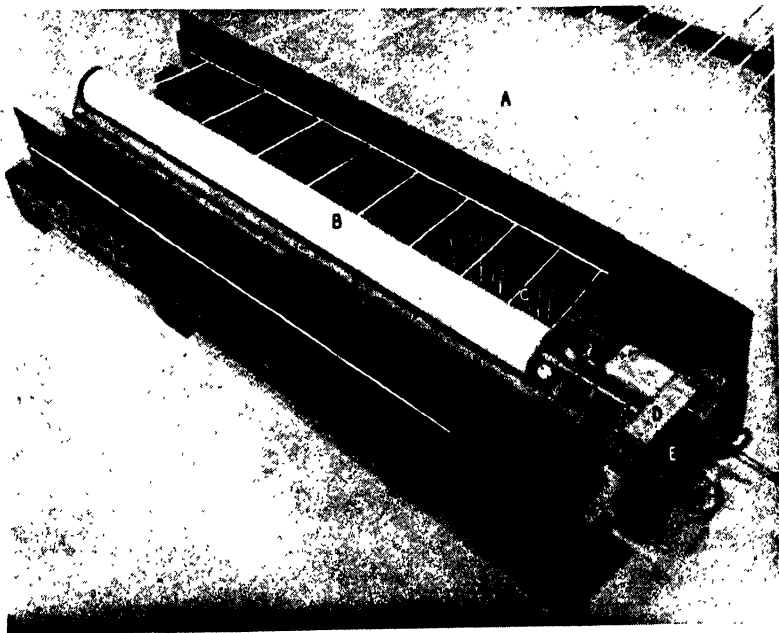


FIG. 1. —Photokymograph for automatically recording auxin curvatures in *Avena*. An intermittent beam of light, *A*, passes through the horizontal slit, striking the photographic paper on the rotating drum, *B*. Projections of the coleoptiles, *C* (straws inserted in place of primary leaf) interrupt the beam, leaving a photographic record of their positions. Speed of rotation of drum *B*, which is driven by hour-hand of electric clock, *E*, can be reduced to any desired period by gear box, *D*.

influencing the plants by exposure to light. Such a method is to be found in the use of the photokymograph (fig. 1). In the most satisfactory type yet used, this apparatus consists of a drum covered with photographic paper and rotating on a horizontal axis. A 1 mm. wide beam of light strikes the total length of the drum at the level of its axis at three or six minute intervals. This beam is obtained by

passing the light of a straight vertical filament of an automobile lamp through three successive horizontal slits, the last one being at a distance of 5 cm. from the drum. Thus practically no stray light comes into the room, especially not into the space between the last screen and the drum, where the plants are placed.

Plants of *Avena* are grown in the usual glass holders, which are assembled in rows of twenty-two plants each, a number of rows being placed along each side of the drum. The machine shown in figure 1 is large enough to accommodate 176 plants. The agar blocks with auxin are put on one side of the cut surface of the decapitated coleoptiles so that the coleoptiles will curve in a vertical plane parallel to the axis of the drum. A record of the angular movement of the curving coleoptile is obtained by photographing the displacement of the straw which is inserted in the place of the primary leaf. This straw, a 6 cm. peduncle of Bermuda grass (*Cynodon dactylon*), makes it possible to avoid exposing the plants to white light in taking the photographs, since only a 1 mm. strip 5 cm. above the curving zone of the coleoptile is illuminated. Projecting the moving tip upward 5 cm. by means of this peduncle also gives an enlargement of the displacement through its lever action. This peduncle with its surrounding leaf sheath just fits the coleoptile, can be adjusted in length by pulling it part way out of its leaf sheath, serves as support for the agar block, and does not bend hygroscopically. An alternative method is to force a fine ni-chrome wire into the partially pulled out and decapitated primary leaf. If the light source is a single filament, as in the lamps here described, then a sharp recording is obtained. This wire has the same advantages of lightness and rigidity as the peduncle just described, and is easier to fit into place. Whether the peduncle or the wire is used, it is inserted about 1.5 mm. into the coleoptile, thus attaching it firmly and yet not allowing it to interfere with the curvature by having a rigid body in the coleoptile.

In this way the position of each plant is indicated on the photographic record as an interruption in the black line caused by the light beam (fig. 2). The curving of the plant is shown by a shift of this interruption in successive recordings. The displacement of the projected coleoptile from its original position can be measured quite accurately (a standard shadow mark of a wire in the rack is used as

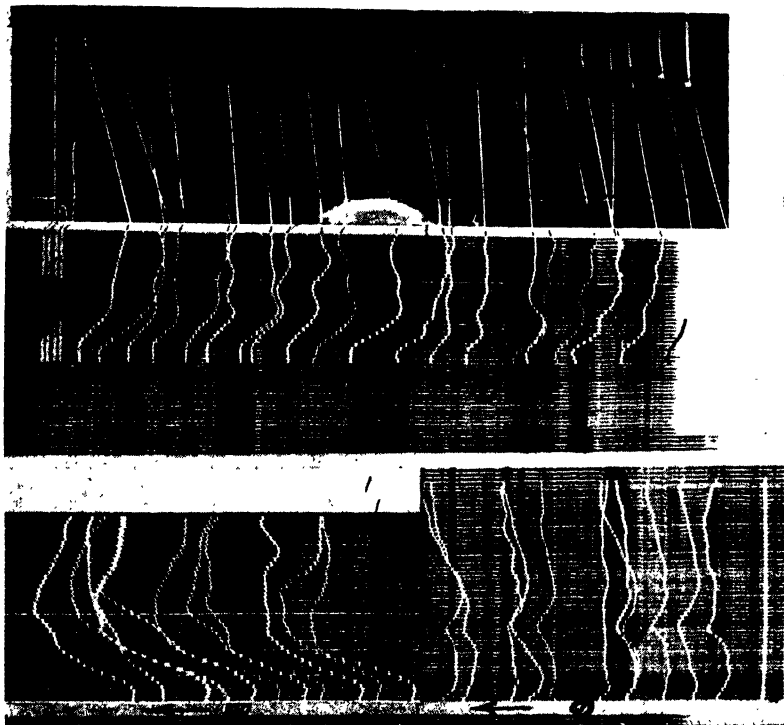


FIG. 2.—A record from the kymograph, with a recording every six minutes. Curvature of coleoptiles shown by displacement of interruptions in black lines in successive recordings. Broad vertical markings at ends of rows are reference marks left by the wire in plant rack. Four treatments shown in this record: in treatment no. 7 a plant has been marked out because of failure of contact between the block and cut surface. Nutations, seen especially in nos. 7 and 8, averaged out in measuring because various wave peaks are out of phase with one another. Arrows indicate direction of negative curvature, which is opposite on opposite sides of drum. Treatments 1, 7, and 8 had low concentration auxin applied, 0.03 mg./l. Treatment 1 had a very short (8 min.) i.d.p. and shows positive curvature; 7 and 8 had a long i.d.p. (220 min.), and show practically no positive curvature. No. 8 had double decapitation and gives more uniform results than single decapitation method of 7; it does not always give so great an increase in sensitivity as in this case. Treatment no. 2 is from another experiment and shows strong curvatures obtained with higher concentrations.

base line), and changes with the tangent of the angle of curvature of the plant. For small angles, 1 mm. corresponds to about  $1^\circ$  of curvature, and as fractions of a mm. can be measured, this method makes it possible to determine small curvatures which cannot be measured in the usual way. At the end of each experiment (usually three to six hours in duration) a shadow picture of the experimental plants is taken to determine whether the contact between agar and plant was good and whether the curvatures were normal.

As will be seen from the photokymograph record (fig. 2), the plants show nutations. These vary in amplitude from test to test, and the various wave peaks for the individual plants occur at different times so that they can be averaged out of the measurements by having several plants in each test. The nutations become less marked as the growth rate decreases, and seem to be the result of a delicately balanced system that is always righting itself and then overshooting the mark so that it becomes necessary to right itself again and again, as in the description of the autotropism of DOLK (2). A similar view was held by GRADMANN (3), who considered nutations as "Überkrümmungen."

As has been determined before (13, 15, 5), there is considerable variation in the sensitivity of *Avena* seedlings to auxin. The variation is of two kinds. First is the variation with age. This could be eliminated from the experiments by always choosing plants of the same age (3 cm. long and grown under the same conditions of light, temperature, and humidity).

Second is the variation with some as yet unknown external conditions. These can be only partly eliminated from the experiments, for they too are of different kinds: hourly variations (24 hour cycle), daily variations (apparently not cyclic), and seasonal variations (yearly cycle). The hourly variations may be eliminated by always having the test plants ready at the same time of day. Not much can be done to avoid the daily variations since they are as yet unpredictable. The annual variations can be eliminated by running the series of experiments within a period of a few days; or, as was actually done in the work for this paper, by repeating the experiments during different times of the year. This was easy to do since the responses happened all to be of the same general form, although of different

magnitudes. The effects of the unpredictable daily variations could also be eliminated by comparing the experiments of several days, since they too were variations in magnitude of response.

The results described in this paper were all obtained under the following conditions: temperature 24° C., humidity 85-90 per cent, and illumination by only occasional red or orange light (from incandescent lamps filtered through a Corning filter 348). The *Avena* seeds (Victory oats), obtained from Sveriges Utsädesförening, Svalöf, Sweden, were dehusked, soaked for one hour in water, and germinated for 30 hours on wet filter paper in dim red light to suppress subsequent mesocotyl growth. Then they were planted in the glass holders and grown in a dark cupboard for 45 hours. About 75 hours after soaking, the coleoptiles were 25-30 mm. long, at which length they were generally used. In the tests a 5-6 mm. tip was removed (unless otherwise stated).

The growth hormone used throughout these experiments was heteroauxin, indole(3)acetic acid obtained from Merck & Co. It was found that a standard solution could be kept for many months by the simple expedient of sterilizing it and keeping it in darkness. Since concentrated solutions lost activity rapidly if kept at high temperatures, the sterilizing process was modified by heating a measured volume of water for the solution (a five gallon bottle with water was heated almost to boiling in a waterbath for several hours), then allowing it to cool to about 70°, at which temperature the auxin crystals were added and the resulting solution mixed and cooled rapidly to room temperature. This sort of sterilizing produced a fully active solution which retained its activity. A siphon and burette previously sterilized with alcohol were then connected, and at any time desired, volumes of an auxin solution of known activity were at hand.<sup>2</sup>

Agar blocks were prepared by cutting 1 mm. slices with a microtome and preserving them in 50 per cent alcohol. Before using, they were washed in running water for one hour and then soaked in the auxin solution to be tested for another hour. They were then ready to be cut into the 7 mm.<sup>3</sup> sized blocks used in the experiments.

<sup>2</sup> While this paper was in press, some stocks prepared in a similar manner lost part of their activity, indicating that further conditions not yet known have also to be controlled.

### III. Results

#### A. AVENA TEST FOR LOW AUXIN CONCENTRATIONS

To test the effect of the time interval between decapitation of the *Avena* seedling and putting on of the auxin-agar block,<sup>3</sup> the following tests were made.

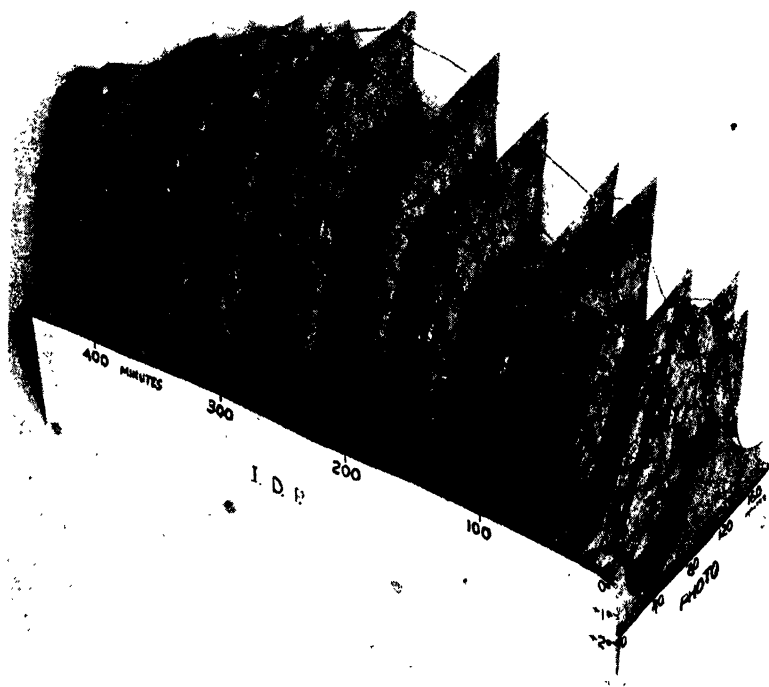


FIG. 3.—Three dimensional model for i.d.p., time after putting block on, and curvature, when a low auxin concentration (0.03 mg./l.) is applied unilaterally. The model is an average of four experiments. Each curve represents the reaction of approximately 30 plants.

At various times after decapitation a low heteroauxin concentration (0.03 mg./liter) was unilaterally applied to decapitated seedlings. The progress of curvature for each i.d.p. was drawn as a graph, and the resulting series of graphs was united into a three dimensional model (fig. 3). The ordinates represent the degree of cur-

<sup>3</sup> The interval between decapitation and putting on is abbreviated as i.d.p. throughout this paper.

vature; the abscissa (to the right) the time in minutes after putting on the agar block; the third axis (toward the left) the time between decapitation and putting on of agar block (i.d.p.). This diagram was made as the average of the four experiments of August 14, 17, 20, 24, 1936; all giving the same form of curves and giving the same absolute curvatures. The diagram applies only to low concentrations, 0.03 mg./l.; for higher concentrations another set of results was obtained and will be described later. This low concentration model shows the following:

1. If blocks are put on soon after a single decapitation, a positive curvature is induced which is later replaced by the negative curva-

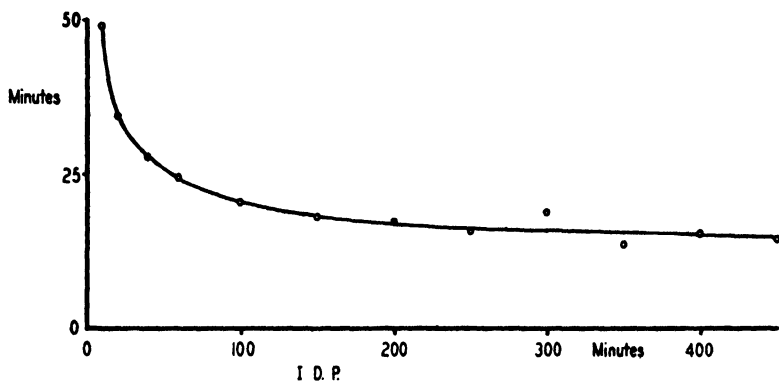


FIG. 4.—Relation between time of application (i.d.p.) of a low auxin concentration (0.03 mg./l.) and time at which growth reaction begins. Each point is an average of 30-100 plants, and is for the single decapitation method.

ture obtained in the standard *Avena* test. This positive curvature may become as great as  $2^{\circ}$  if blocks are put on very soon after decapitation, and decreases to  $0^{\circ}$  as the i.d.p. increases to 100 minutes (fig. 3).

2. The *Avena* test curvature (away from the block) begins at about 50 minutes after putting on if the i.d.p. is short. With increasing i.d.p. this period decreases to a limit of about 17 minutes at an i.d.p. of 100 minutes or greater (fig. 4).

3. If the i.d.p. is less than 50 minutes, the auxin curvature shows a regeneration reaction (regression of curvature, figure 3), which occurs at the regeneration time, 130 minutes after decapitation. (Al-



though regeneration time is usually given as 150 minutes, if the regression of curvature is taken to be the regeneration time, in these experiments regeneration time is 130 minutes after decapitation; factors affecting regeneration time are described below; III D<sub>3</sub>, III E<sub>5</sub>, IV 3a.) For i.d.p. of greater than 50 minutes there is also a regeneration-like reaction which occurs at 80 minutes after applying the auxin (fig. 5). This pseudo-regeneration reaction will be considered later (III A<sub>7</sub>cd; III D<sub>3</sub>; III E<sub>5</sub>).

4. The sensitivity of the seedlings to low auxin concentrations increases as the i.d.p. increases, rapidly for the first 30 minutes, gradually from 30 to 240 minutes, and finally decreases after 240 minutes (fig. 3).

5a. For the first 100 minutes of i.d.p. the curvature has a general procedure as follows: a positive curvature (toward the agar block), a succeeding negative curvature (curvature of the *Avena* test), then a temporary regression of this negative curvature (fig. 3).

5b. At about 100 minutes i.d.p. the negative curvature begins, without a preliminary positive curvature, at 17 minutes after putting on, and continues rapidly until 70 minutes after putting on, then continues less rapidly (fig. 3).

5c. For i.d.p. greater than 100 minutes a strong negative curvature begins at 17 minutes after putting on and continues until 80 minutes after putting on. A temporary regression of this curvature follows with a final gradually increasing negative curvature (fig. 3). With increasing i.d.p. this temporary regression decreases until it becomes almost unnoticeable at 300 minutes, and thereafter increases again (fig. 3).

6. The maximum rate of curvature was plotted (in degrees of curvature per hour) against length of i.d.p. It increased rapidly for the first 100 minutes, increased less rapidly from 100 to 250 minutes, and finally was constant after 250 minutes (fig. 7).

7a. The standard *Avena* test (13, 14) has two decapitations an hour apart; after the second decapitation the auxin-agar blocks are put on. According to VAN DER WEIJ, the second decapitation increases the sensitivity of the test plants to auxin because it postpones regeneration of the physiological tip. In order to test this contention, tests with decapitations spaced so that there was one de-

capitulation just before putting on and so that the plants never remained for more than two hours without decapitation (three decapitations spaced two hours apart were used by HEYN (4) to get

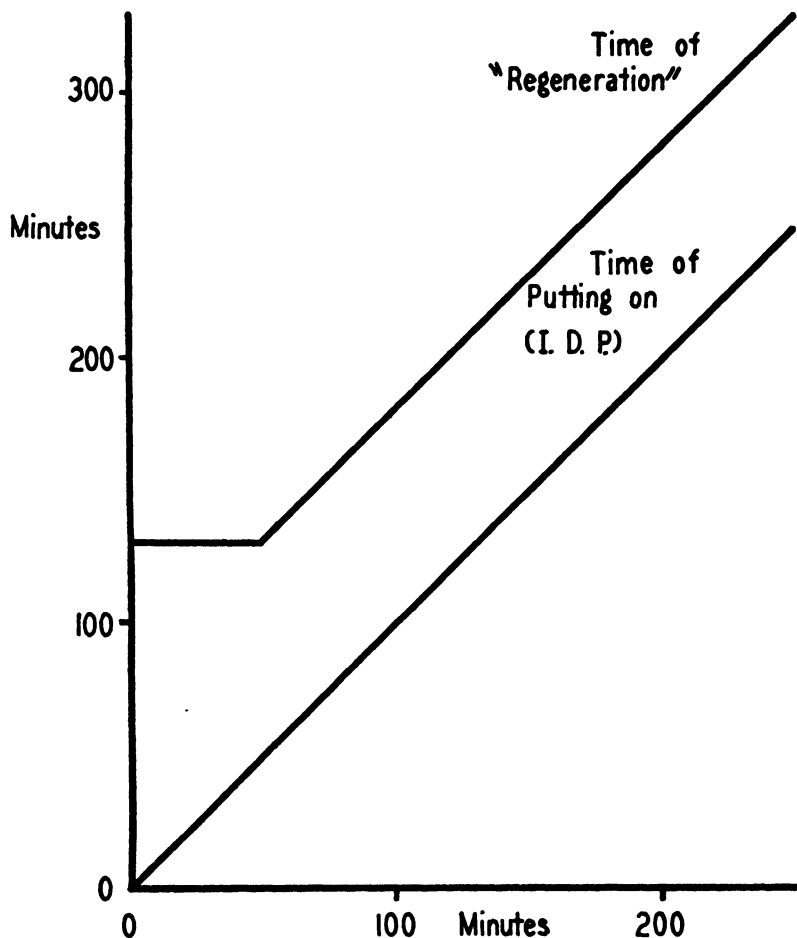


FIG. 5.—Regeneration (regression of curvature) time in relation to i.d.p. for low auxin concentration. Schematic curve from experiments of figure 3.

direct proportionality between auxin and curvature), were run at the same time as the single decapitation tests mentioned previously and with the same i.d.p. as those tests. It was found (for both low and high concentrations) that the first and last decapitations were

by far the most important, so that eventually the intervening ones were omitted.

7b. For each i.d.p. except 100 minutes the procedure of the curvatures was of the same form for both methods. For this auxin concentration, however, curves for i.d.p. greater than 60 minutes showed somewhat greater sensitivity for the two decapitation method (fig. 6).

7c. The two decapitation curve for the 100 minute i.d.p. showed a small regression at about 80 minutes after putting on, while the

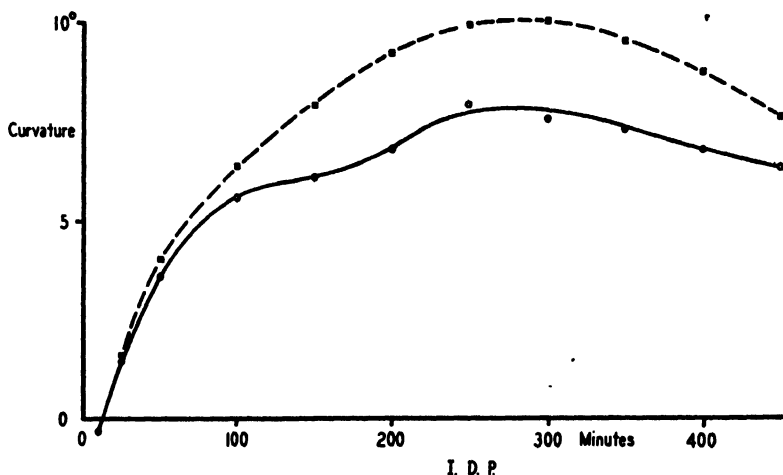


FIG. 6.—Sensitivity of *Avena* to low auxin concentration, 0.03 mg./l., with increasing i.d.p. Solid line for single decapitation (eleven experiments) and broken line for double decapitation method. Measured at 80 minutes after putting on.

single decapitation curve for the same period, as described above (III A5b), showed no such regression. All other curves also showed a somewhat greater regression or tendency toward regression for the two than for the one decapitation method.

7d. On the basis that a second decapitation postpones regeneration (VAN DER WEIJ), it would be expected that for any i.d.p. up to regeneration time, a second decapitation just before putting on would postpone the “regeneration” reaction (regression of curvature) by the length of time between the two decapitations. This was not found to be the case; the reaction was not postponed at all.

7e. The maximum rate of curvature was greater for the two decapitation method than for the single one (fig. 7).

Except for very short i.d.p. (which are generally not used in the *Avena* test), it will be seen from figure 3 that the main curvature for low applied auxin concentrations is completed after about 80 minutes, and after that time may even show a regression. Since this is even more true for the double decapitation method, it is evident that for low auxin concentrations the standard *Avena* test may be ad-

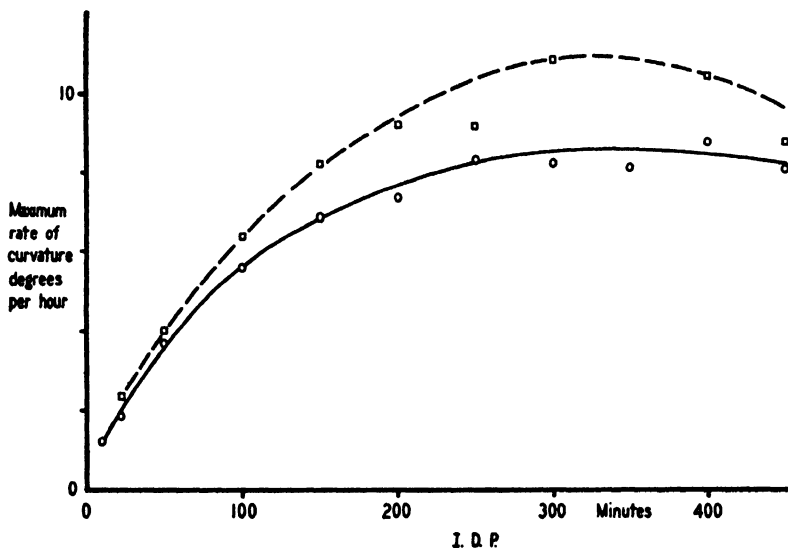


FIG. 7.—Greatest rate of curvature in relation to i.d.p. for low concentration, 0.03 mg./l. Data from same eleven experiments as figure 6. Solid line for single and broken line for double decapitation.

vantageously modified by taking photographs at 80–90 minutes after putting on.

#### B. THE AVENA TEST FOR HIGH AUXIN CONCENTRATIONS

Figure 8 shows the relation between i.d.p. and procedure of curvature for a high applied auxin concentration giving the so-called maximum angle when the single decapitation method is used. The results are distinctly different from those for low applied auxin concentration (fig. 3).

1. If the i.d.p. is very short, the curvature starts soon after application of the high concentration auxin blocks (about 20 minutes) and proceeds at approximately the same rate for more than three hours.

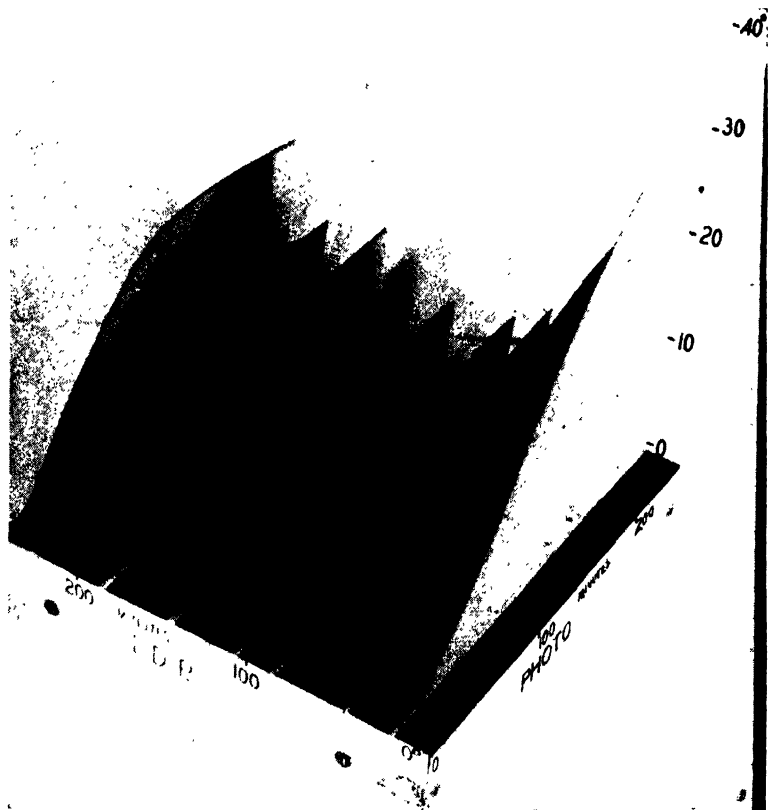


FIG. 8.—Three dimensional model for i.d.p., time of photographing, and curvature, when high auxin concentration (0.5 mg./l.) is applied. Model made from one of three experiments giving same general results. It shows increasing maximum angle with decreasing i.d.p. (for i.d.p. of less than 130 minutes).

2. With increasing i.d.p. the rate of curvature decreases, but the form of the curve is about the same until 130 minutes i.d.p., when a minimum reaction is reached.

3. Beyond 130 minutes i.d.p. the maximum rate of curvature increases again, but the form of the curve is different in that the rate

of curvature decreases sharply or may be slightly reversed at about two hours after putting on.

4. A second decapitation just before putting on greatly increases the curvature with high concentrations (fig. 9); for i.d.p. longer than 130 minutes this increase is about 100 per cent. The concentration

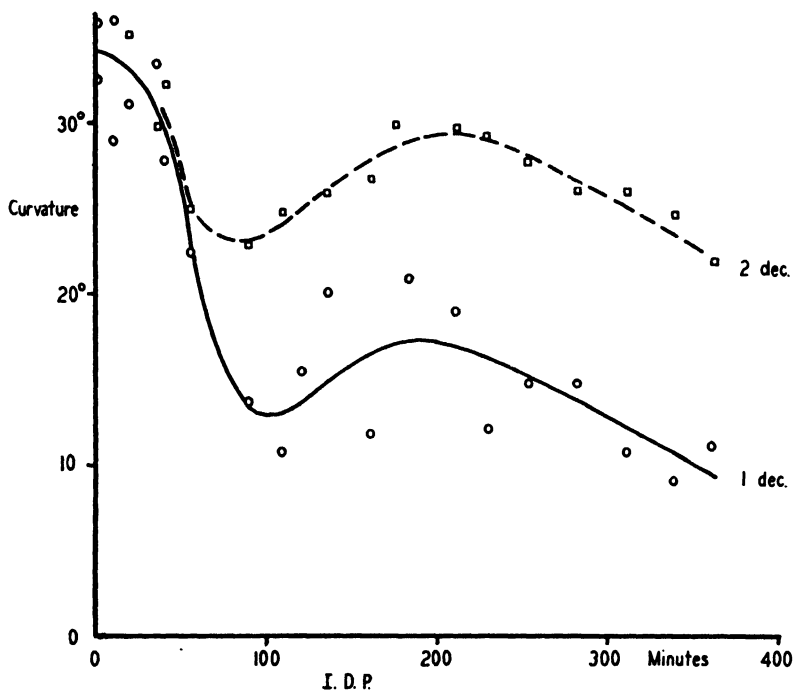


FIG. 9.—One of three similar experiments, showing complex effect of increasing i.d.p. on maximum angle for both single and double decapitation method. For longer i.d.p. double decapitation method gives greater uniformity of results and about twice as high a response.

giving maximum angle is nearly the same for the one and two decapitation methods (fig. 11).

5. To test the explanation that has been given for this increase in sensitivity after a second decapitation (13), namely, that the plant is more nearly empty of auxin because regeneration has been postponed by the second decapitation, the following experiments were conducted.

Seedlings were decapitated at three hours after a first decapita-

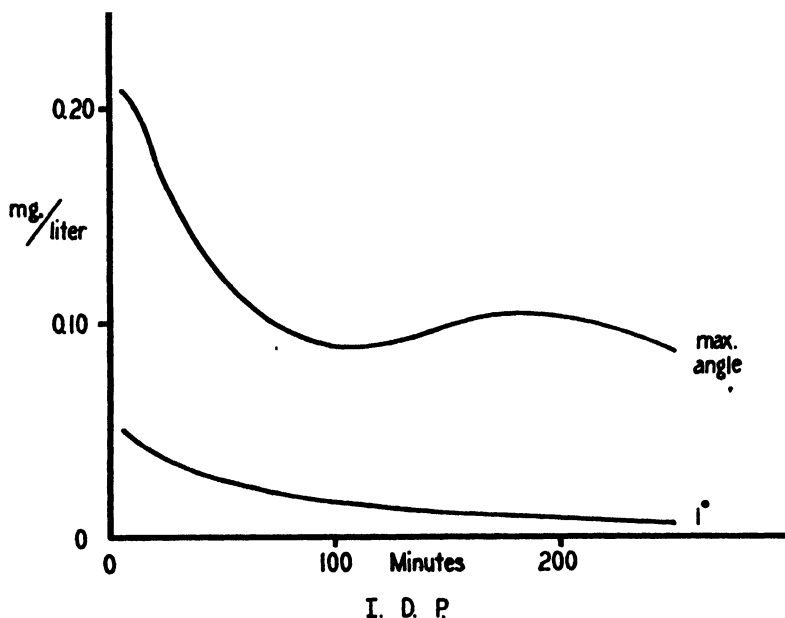


FIG. 10.—Range of concentrations over which proportionality is obtained with increasing i.d.p. Upper curve shows lowest concentration that gives maximum angle at any particular i.d.p., and lower curve shows concentration giving  $1^\circ$  of curvature.

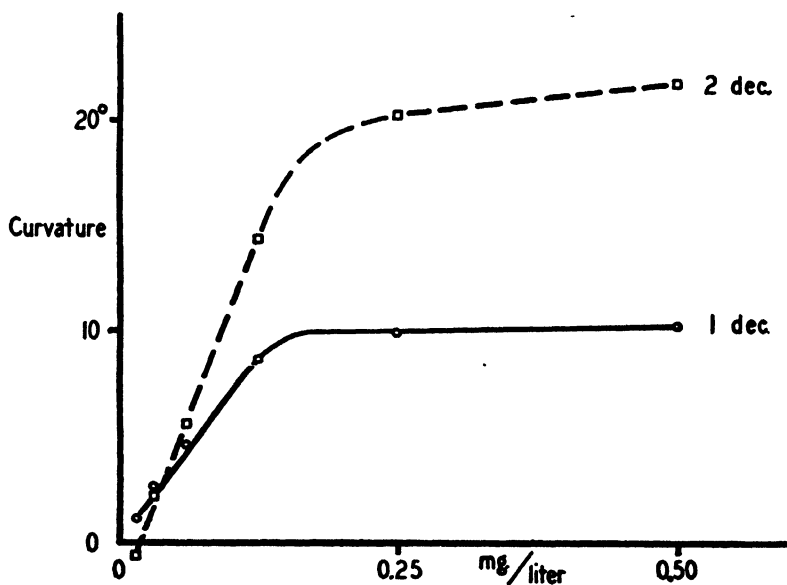


FIG. 11.—Effect of second decapitation just before putting on (broken line) on direct proportionality of curvature to applied concentration of auxin (i.d.p., 150 minutes, photographed at 80 minutes).

tion and a maximum angle concentration of auxin-agar was applied symmetrically to the cut surfaces of two sets of seedlings, one of which was decapitated a second time, the other not. Two marked 5 mm. zones below the cut surface (giving a total of 10 mm., most of the zone over which curvatures occur within two hours) were measured by means of a horizontal microscope to determine growth. Both sets gave the same growth (table 1). Controls without applied

TABLE 1

DIRECT GROWTH MEASUREMENTS SHOWING THAT SECOND DECAPITATION JUST BEFORE PUTTING ON DOES NOT CHANGE MAXIMUM GROWTH AFTER AUXIN APPLICATION BUT CHANGES THE RESPONSE TO AUXIN. FIGURES ARE AVERAGES OF TEN PLANTS

TREATMENT	ZONE	GROWTH IN MM.	
		ONE DECAPI-TATION	TWO DECAPI-TATIONS
Auxin-agar (0.25 mg./l.) symmetrically applied	First 5 mm. zone	0.60	0.55
	Second 5 mm. zone	0.55	0.60
	Top zone of 10 mm. (total)	1.15	1.15
Controls without applied auxin or agar	First 5 mm. zone	0.35	0.15
	Second 5 mm. zone	0.45	0.25
	Top zone of 10 mm. (total)	0.80	0.40
Effect of auxin on first 10 mm. zone.....		0.35	0.75

auxin or agar were also measured at the same time and gave the results seen in the table.

From table 1 it is clear that for the one decapitation method the greatest growth difference that can be obtained on the two sides of a coleoptile when maximum angle concentration auxin is applied to one side is 0.35 mm., because the regenerated tip is producing auxin on the opposite side also. For two decapitations this difference is 0.75 mm., since the second decapitation stops regeneration and leaves the side opposite the agar block with only its residual auxin.



The relative difference of the two sides is about 1 to 2, the same as found for maximum angle.

It might be predicted further that since concentrations above 0.25 mg./l. (maximum angle) all give maximum angle, they should also give the same growth as the concentration 0.25 mg./l. Table 2 shows that they do not. DU BUY and NUERNBERGK (1) have ex-

TABLE 2

DIRECT GROWTH MEASUREMENTS SHOWING THAT SUPRA-MAXIMUM ANGLE CONCENTRATION GIVES SOMEWHAT GREATER GROWTH THAN MAXIMUM ANGLE CONCENTRATION. FIGURES ARE AVERAGES OF TEN PLANTS

CONCENTRATION OF AUXIN IN AGAR SYMMETRICALLY APPLIED		GROWTH IN MM.	
		ONE DECAPI- TATION	TWO DECAPI- TATIONS
0.25 mg./l.	First 5 mm. zone	0.60	0.60
	Second 5 mm. zone	0.65	0.55
	Top zone of 10 mm. (total)	1.25	1.15
0.75 mg./l.	First 5 mm. zone	0.75	0.70
	Second 5 mm. zone	0.75	0.75
	Top zone of 10 mm. (total)	1.50	1.55

plained this effect by lateral transport of auxin with such high concentrations.

### C. PROPORTIONALITY BETWEEN AUXIN CONCENTRATION AND CURVATURE

In view of the very different behavior of the test plants to low and high auxin concentrations when the period between decapitation and putting on of the agar block increases from a few minutes to several hours, the intermediate concentrations had to be investigated as well. This meant an investigation of the relation between concentration and curvature for different i.d.p.

Six experiments were conducted at different times of the year, all giving the same results. A series of auxin concentrations, covering

the whole range between just measurable activity and maximum angle, were put on at increasing i.d.p. (fig. 12).

1. At all i.d.p. the relation between curvature and concentration is a straight line for concentrations below maximum angle.

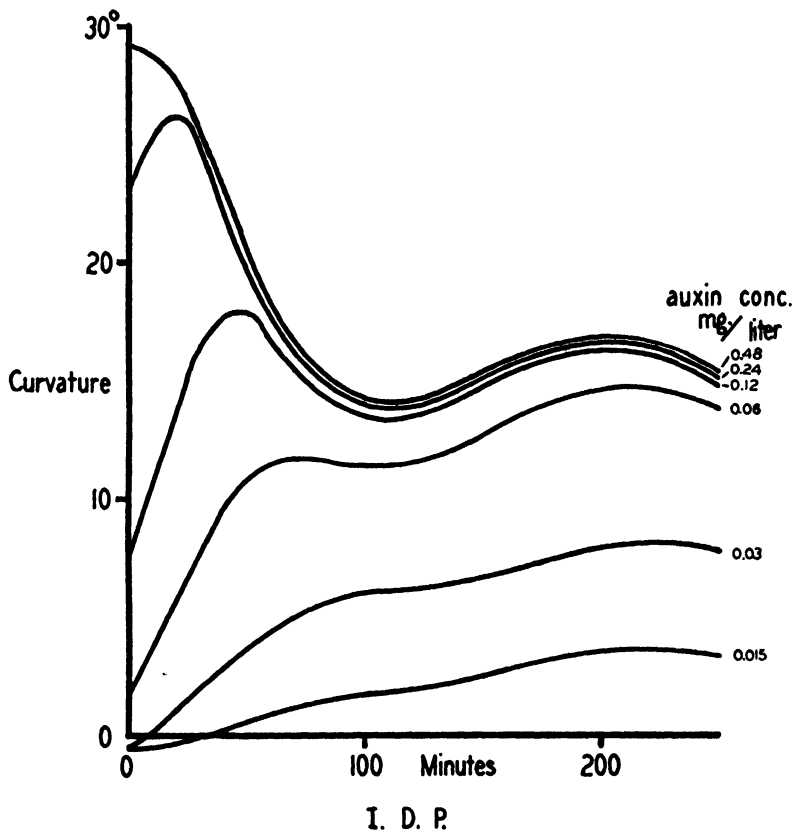


FIG. 12.—Relationship of curvature and i.d.p. for different auxin concentrations and a single decapitation. Schematic curves drawn from combined results of many experiments. Measured at 80 minutes after putting on.

2. For short i.d.p., however, the straight line transects the abscissa to the right of the origin. With increasing i.d.p. the line transects the abscissa nearer to the origin, until after 2–3 hours (this period varies from day to day) direct proportionality between curvature and concentration obtains.

3. For long i.d.p. (3-4 hours), the test plants show a maximum sensitivity (fig. 6), but the range of concentrations that can be determined quantitatively is shifted and is narrower than for short i.d.p. This can be seen by referring to figure 10, which shows that both the maximum and the minimum concentrations which can be tested are lowered by increasing i.d.p.

4. Figure 12 shows that, within limits, the sensitivity for all concentrations of auxin increases with increasing i.d.p.

5. Blocks 1 mm.<sup>3</sup> and 7 mm.<sup>3</sup> were used in the *Avena* test to see whether the size of blocks affected the direct proportionality of curvature to applied auxin concentration. Both sizes gave the same curvatures if photographs were taken at 80 minutes after putting on, but if they were taken at 120 minutes the 7 mm.<sup>3</sup> blocks gave a somewhat greater response than the 1 mm.<sup>3</sup> blocks. For both cases the curvatures were proportional to the applied auxin concentration, and became more nearly directly proportional as the i.d.p. increased, finally reaching direct proportionality at an i.d.p. of about three hours.

6. Comparison of the one and two decapitation methods over a range of concentrations (long i.d.p.) shows that both give a straight line curve (fig. 11), the two decapitation method giving higher angles for high concentrations and slightly lower angles for extremely low concentrations. This results in the one decapitation method giving more nearly direct proportionality, although both methods approach it closely for i.d.p. of about three hours. For extremely low concentrations (0.01 mg./l.), with a long i.d.p. (three hours), the two decapitation method has a tendency toward positive angles. Under these conditions the two decapitation method gives values 1.5° to 2° lower than the single decapitation method. This is just the amount of the positive curvature which precedes the negative one if auxin-agar is applied immediately after decapitation (III A 1).

It should be pointed out that for very low concentrations the single decapitation method 3-4 hour i.d.p. is best. For moderate concentrations the double decapitation method and 3-4 hour i.d.p. works best because it gives higher angles (which can be measured more accurately) and gives more uniform results.

7. Since (1) the moment at which the plants start to curve is independent of the applied auxin concentration, (2) the curvature is

proportional with the concentration at 80 or 90 minutes after application, and (3) the rate of curvature is constant for about an hour, it follows that at any time after curvature has set in it is proportional with the concentration (for example, see 17, figure 21).

#### D. EFFECT OF LENGTH OF TIP REMOVED

Among the other factors affecting the *Avena* test, the length of the tip which is cut off is important.

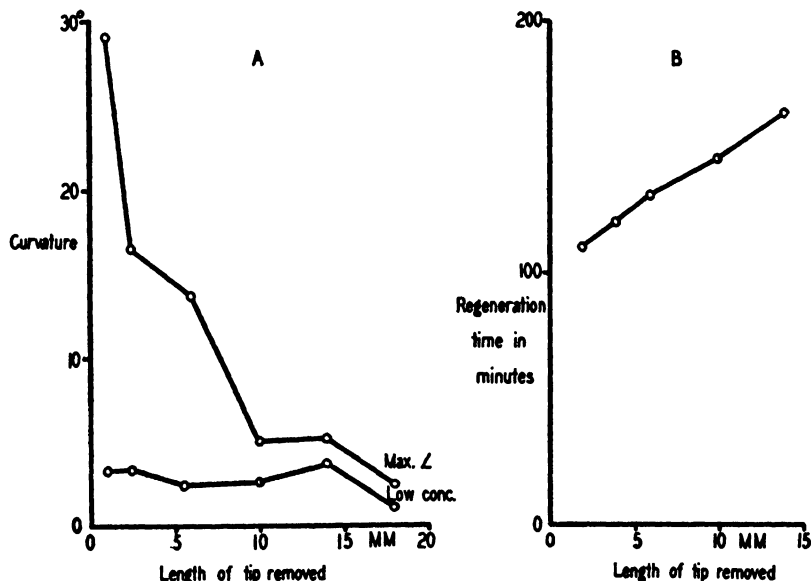


FIG. 13.—(a) Effect on maximum angle and low concentration curvatures caused by removing increasingly longer lengths of tip when decapitating. (b) Effect on regeneration (regression of curvature) time caused by removing increasingly longer lengths of tip at decapitation. Data are from one of two experiments which gave similar results.

1. As greater lengths of tip are removed, the maximum angle decreases continuously (fig. 13a). The rate of curvature is changed, but the procedure of the curvature is otherwise independent of the length of tip removed.

2. With lower concentrations, however, the initial rate of curvature is less dependent on the length of the removed tip (fig. 13a), except for old plants where the rate of curvature falls with increasing length of removed tip.

3. With low concentrations it can be seen that the length of removed tip has a great effect on the "regeneration" time; the greater the length removed the longer the period before the curvature starts to recede (fig. 13*b*). This is in agreement with the observations of LI (6), who found that the moment of regeneration, as measured by the sudden increase in growth rate of decapitated coleoptiles after the initial gradual drop, is delayed when increasing lengths of tip are removed. Thus, for short i.d.p. it is confirmed that the regeneration of the physiological tip is responsible for the dip in our curves at regeneration time.

#### E. FLOODING WITH AUXIN

Since we know that decapitation greatly affects the auxin content of the plants, experiments were carried on in which this content was controlled. To this end a dab of lanolin paste containing auxin was applied to the intact tip for two hours preceding the experiment, thereby increasing the auxin content of the plants above normal. Tests were then run with high and low auxin concentrations.

1. The reaction of low auxin concentrations is decreased compared with untreated plants (fig. 14*a*).

2. More remarkable is the effect on the maximum angle, which first decreases, but with very high auxin concentration again increases (fig. 14*a*). For these high auxin concentrations, however, the curved zone is very short and near the tip. When the auxin concentration was decreased below normal by decapitation and regulated by symmetrical application of auxin-agar to the cut surface, similar results were obtained.

3. There was a greater decrease in sensitivity to low concentrations as the auxin concentration in the plant was increased (fig. 14*b*), than for the paste method above.

4. There was a sharp decrease in sensitivity to high concentration (fig. 14*b*) without the final increase in sensitivity as for the paste method above, probably because the highest concentration applied in the agar was not comparable with the highest applied in the lanolin.

5. The regeneration time increased as the internal auxin concentration increased (fig. 14*c*).

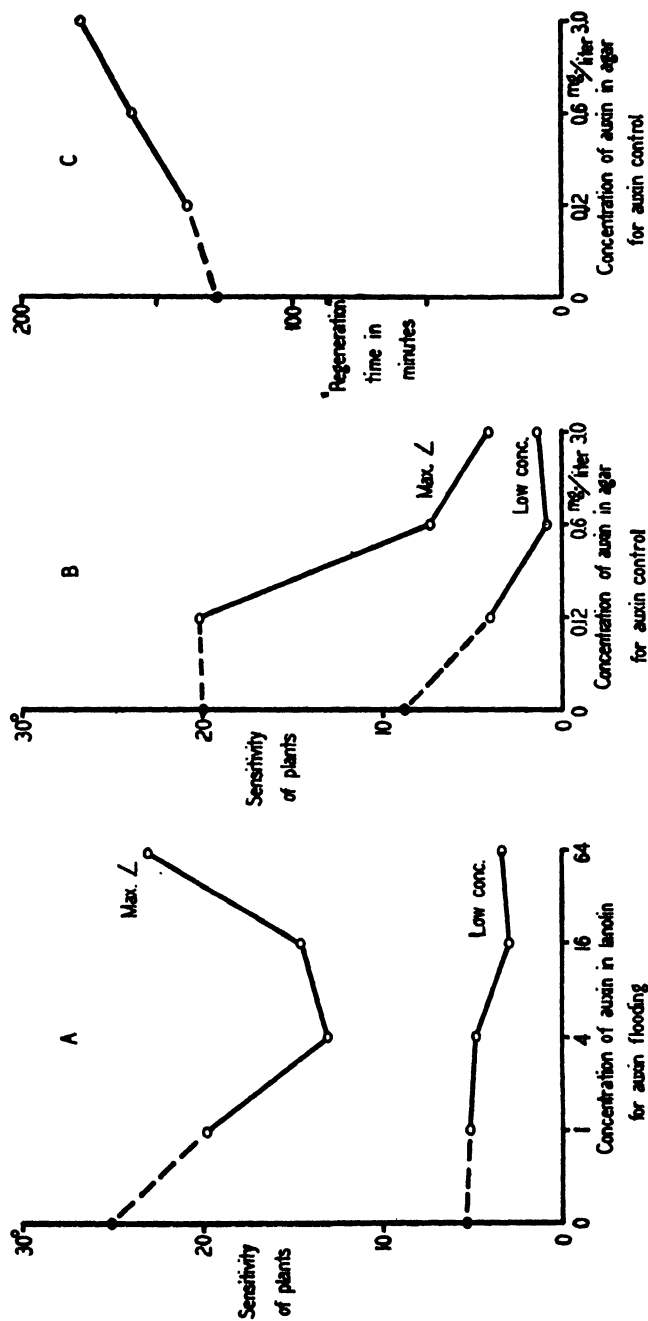


FIG. 14.—(a) Effect on maximum angle and low concentration curvatures caused by increasing auxin content (auxin flooding) of intact coleoptiles with lanolin paste. (b) Effect on maximum angle and low concentration curvatures caused by changing content of coleoptile by decapitating and symmetrically applying agar and auxin-agar. (c) Effect on regeneration (regression of curvature) time caused by changing auxin content of decapitated coleoptile with agar and auxin-agar, both for low and high applied auxin concentrations. Curves drawn from single experiment; a second experiment gave similar results.

#### IV. Discussion

1. The positive curvature that occurs when agar blocks of low auxin concentrations are put on soon after a first or second decapitation is not yet explained. It exists, however, and cannot be the result of a defective test method because it does not occur when the interval between the last decapitation and putting on is great.

This positive curvature can hardly be caused by loss of auxin into the block because all attempts to get auxin into such blocks resulted only in the obtaining of auxin precursor. It varies in magnitude from day to day, and it is of about the same magnitude for all lengths of tip removed (measurements were made on plants having tips removed varying from 2 to 18 mm.). It seems that the conditions under which these positive curvatures appear are those under which the onset of negative curvature is late.

2. The negative curvatures are of course caused by the influence of auxin on growth, since auxin is a limiting factor for growth in the test plants. Because a test plant having much auxin is already growing at nearly its maximum rate, it is to be expected that additional auxin could not raise the growth rate very much. This is shown to be the case (III E 1-4) where artificially increased auxin content of the test plants lowered the sensitivity to unilaterally applied auxin of low and high concentrations, and (III B 5) where direct growth measurements were made on plants having high and low auxin content by allowing or preventing regeneration. The effect of high auxin paste, however, is an exception not explained in this way.

Since after decapitation the auxin content drops off first rapidly and then more slowly, we should expect that as the i.d.p. increases, the sensitivity to applied auxin would increase first rapidly and then more slowly, which is what has been found for low concentrations (III A 4) and high concentrations (III B 3; III C 4; III B 5).

From this it would be expected that with decreasing i.d.p. the maximum angle would steadily decrease, but for the shorter i.d.p. this decrease is offset by the increased range of sensitivity (figs. 10, 12). Thus the relation between maximum angle and i.d.p. is a com-

plex one, showing two maximums for both the one and two decapitation methods.

3a. From the procedure of the curvatures with low applied auxin, the regression of the curvature for very short i.d.p. must be connected with the regeneration of the physiological tip on the side of the coleoptile opposite the agar block. (The side with the agar block produces less auxin by regeneration because precursor is removed into the agar block, 11.) Consistent with the explanation that this regression is predominantly a regeneration effect is that for i.d.p. up to 50 minutes it always occurs 130 minutes after decapitation; that is, at regeneration time, regardless of when the auxin is applied (III A 3; fig. 5).

Regeneration is also an important factor in the *Avena* test when high concentrations are applied, and its effects can be eliminated by a second decapitation just before applying the agar blocks, thereby obtaining greater sensitivity to high concentrations (III B 4, 5). There is therefore a regeneration effect on sensitivity and also a regeneration effect on procedure of curvature with low applied auxin concentration and short i.d.p. But we see also that the apparent regeneration effect (regression of curvature) on procedure of curvature with low applied auxin concentration and i.d.p. of longer than 50 minutes must be a result of something other than regeneration.

3b. For i.d.p. longer than 130 minutes, regeneration will have occurred before the agar block was put on and therefore cannot explain the sudden change in rate of curvature at 80 minutes after putting blocks on. There are some other possible explanations of these changes in rate of curvature: (1) Geotropism can be ruled out because the break in the curve occurs even with angles too small to produce geotropic perception in the periods involved, even in intact plants. (2) Aging is ruled out by the suddenness of the break and by the increase in sensitivity for longer i.d.p. which could not occur if aging were a significant factor. (3) This leaves as a third possible explanation, which may be tentatively suggested, the food factor and its transport under the influence of auxin. It has been shown (16) that no satisfactory explanation of the effects of auxin can be



given without considering the relation of auxin to other growth factors, in this case the food factor. The food factor moves upward in the coleoptile, perhaps under the influence of auxin, and also seems to be used up during growth. When auxin induces growth in the coleoptile the food factor will be used up so that eventually food factor must limit growth, and thereby limit the rate of curvature. This is similar to the explanation of autotropism by DOLK (2).

### V. Summary

1. With an automatic photokymograph, the procedure of curvature in the *Avena* test was investigated. In general, when auxin was applied unilaterally there was little or no effect for about 20 minutes, after which curvature set in and continued at a constant rate for about an hour, then diminished or reversed, depending on the treatment.

2. It was found that increasing the auxin concentration inside the plant decreased the sensitivity to unilaterally applied auxin. This explains the increase in sensitivity after decapitation, since the auxin content decreases after removal of the tip.

3. To study this process in detail, the effect of the i.d.p. (length of interval between decapitation and putting on of the agar block) and the number of decapitations was followed for different concentrations of auxin.

4. For all concentrations up to maximum angle concentration, the sensitivity increased rapidly for the first 30 minutes i.d.p., increased gradually until 200–240 minutes i.d.p., then gradually decreased.

5. But the maximum angle concentration fell off rapidly for the first 100 minutes i.d.p.

6. For i.d.p. longer than 100 minutes, a second decapitation just before putting on causes: (a) the reaction to maximum angle concentration to be doubled; (b) the reaction to extremely low concentration to be slightly decreased, even to the extent of sometimes giving small positive angles; (c) more uniform reactions.

7. A modified standard *Avena* test is recommended: (a) an i.d.p. of 3–4 hours; (b) a second decapitation 20–40 minutes before putting on; (c) photographs at 90 minutes after putting on.

8. Besides the regeneration effect which reverses the curvatures at 130 minutes after decapitation for i.d.p. of less than 50 minutes, there is also a pseudo-regeneration effect for longer i.d.p. which also balances or reverses curvature at 80-90 minutes after putting on.

9. This effect, and certain others (decreases of maximum angle concentration with increasing i.d.p., and positive angles if low concentration auxin-agar blocks are put on soon after decapitation) cannot be explained by geotropism, lateral auxin transport, or aging. Thus it is tentatively proposed that they may be due to food factor distribution

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# DEVELOPMENT AND ANATOMY OF PRIMARY STRUCTURES IN THE SEEDLING OF CUCURBITA MAXIMA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 488

A. GERALDINE WHITING

(WITH SIX FIGURES)

## Introduction

Since HARTIG (14) discovered the sieve tube and internal phloem in *Cucurbita pepo* in 1854, numerous studies have been made of the anatomy of the Cucurbitaceae. The investigations have usually been incidental to larger comparative surveys with emphasis on ontogeny and phylogeny. They show a haphazard distribution among the different genera, and at the same time fail to indicate the interrelationships of the various phases studied. The present paper gives a more complete description of the development and the histological anatomy of the seedling of a single species.

After the work of HARTIG, VON MOHL (29) made similar observations on the phloem in several plants, including *Cucurbita pepo*. To both investigators the significance of this tissue was admittedly unknown. Later, when the physiological and cellular nature of phloem was better understood, FISCHER (10) made an extensive study of this tissue in the Cucurbitaceae, noting its character and its unusual distribution. BRAEMER (3), studying the drug plants *Bryonia dioica*, *Citrullus colocynthis*, and *Ecballium elaterium*, differed with FISCHER in regard to the interpretation of certain tissues which the latter called phloem and which BRAEMER considered a segmented lactiferous system quite distinct from phloem.

Meanwhile DE BARY (8) originated the term bicollateral for this type of bundle with internal and external phloem. He cited the Cucurbitaceae as the type. This formulation of a term precipitated a controversy as to whether the internal phloem is actually part of the bundle or whether it is independent and the bundle therefore not bicollateral. Various investigators (15, 21, 25, 12, 1, 28, 5, 30, 19)

have affirmed or denied the direct relationship of this inner phloem to the vascular bundle.

Other anatomical aspects of the Cucurbitaceae also have been studied. The root tip was described by JANCZEWSKI (17). The root itself was studied by VAN TIEGHEM (26) and by RUTLEDGE (24). JANCZEWSKI (18) and VAN TIEGHEM with DOULIOT (27) traced the development of lateral roots but disagreed completely in their interpretations. The transition has been reported by various workers. GÉRARD (13) briefly described it in *Cucurbita maxima*. Later DANGEARD (7) gave a short account, agreeing with GÉRARD, but the following year LAMOUNETTE (21) presented a somewhat different interpretation for the same species. RUTLEDGE (24) has given a more detailed description of the transition in this species. Others (10, 30, 19) have mentioned the transition but only as incidental to some other study. The peg specifically has been studied by FLAHAULT (11), NOLL (23), CROCKER, KNIGHT, and ROBERTS (6), and by others.

In the hypocotyl the primary structure of the bundle in general has been described by the investigators involved in the bicollateral-ity controversy. Although most of their work is based on the upper axis or stem derived from the epicotyl, much of it may be applied to the bundles in the hypocotyl. In addition to those already mentioned, HOLROYD (16) and ZIMMERMANN (31) have referred to primary structures for a number of different genera, including *Cucurbita*. The course of the bundle in the hypocotyl, through the cotyledonary node and into the cotyledons, has been vaguely mentioned by DANGEARD (7) for several genera, not including *Cucurbita*, and by JEAN (19) for *C. pepo*. The relationship of the epicotyl to this lower axis has been overlooked, except for an incorrect interpretation by DANGEARD (7) and a description of the lower two or three internodes of the stem by MANTEUFFEL (22).

### Material and methods

In the present study observations were limited to *Cucurbita maxima* Duchesne. This species is probably subtropical and American in origin (9). As listed by CASTETTER and ERWIN (4), many horticultural varieties have been developed; that used for the present work was the winter squash, Blue Hubbard. For a study of the

seedling, several plantings were made for daily collection up to seven or eight days. Older material was collected approximately each week during the first half of the growing season. All the material was grown under ordinary garden conditions except that the bases of the older plants were protected by wire cages against the destructive activity of the squash vine borer, *Meletittia satyriniformis* Hübner. In fixing the material, a modification of Navashin's solution and a chromacetic solution were used. The material was cut at various thicknesses: at 6  $\mu$  for the study of the origin of lateral roots, at 10  $\mu$  for transverse sections of young material, at 15  $\mu$  for older material. Longitudinal sections were more satisfactory at 20  $\mu$  for xylem but at about 10  $\mu$  for phloem. Flemming's triple stain was used, also light green and fast green with safranin. Gourley's method for staining and clearing the vascular system, and a slight modification, produced excellent material for tracing the course of the vascular bundles in the various organs of the plant.

### Investigation

#### SEED AND SEEDLING

The seed of *Cucurbita maxima* is characteristically dull white, flat, and elongated or broadly oval with a strong marginal rim which is interrupted at the micropylar end. The anatomy of the seed has been described by BARBER (2) and KONDO (20). In planting, the seed lies flat. Under favorable conditions, at the end of two days the root has protruded between the halves of the seed coat and turned at right angles downward in the soil (fig. 1A). The growth of the root is rapid; by the next day it may be 3 cm. in length. This same day, the third day (all dates have reference to the time of planting), the peg begins to develop as a small lateral ridge flattened against the hypocotyl and placed in the angle formed by the horizontal hypocotyl and the perpendicular root (fig. 1B). In one or two specimens this outgrowth extended farther around the axis. On the fourth day many lateral roots show just below the peg, and the peg itself has enlarged considerably. At the same time the hypocotyl begins to elongate upward but the cotyledons remain horizontally within the seed coat. The latter soon begins to split, because the lower half is firmly held in place by attachment to the lower surface of the hori-

zontal peg and the upper half is being forced away by the upward growth of the arched hypocotyl (fig. 1C). Finally the seed coat splits sufficiently and the cotyledons are drawn out. The recurved hypocotyl with the cotyledons breaks above the soil level about the sixth day, following which the seedling soon becomes fully erect.

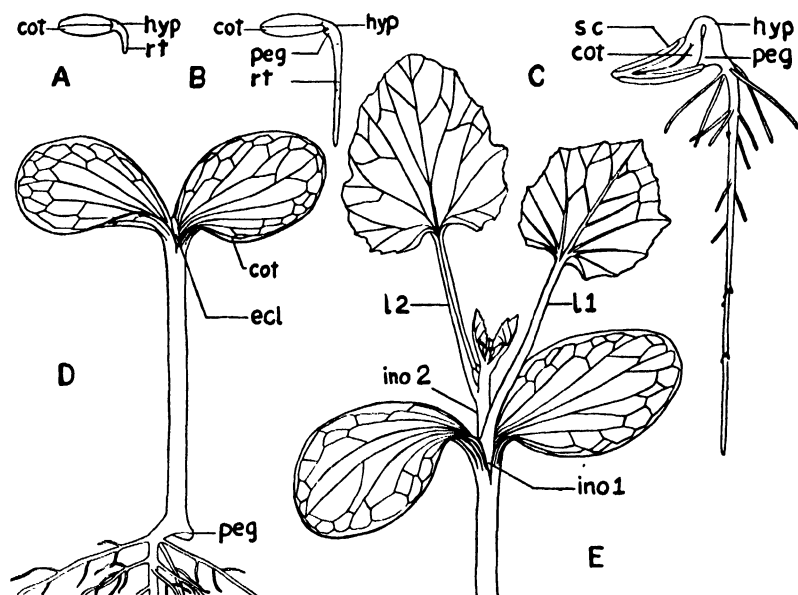


FIG. 1.—Development of seedling: *A*, two days, axis before formation of peg, seed coat removed (*cot*, cotyledon; *hyp*, hypocotyl; *rt*, root); *B*, three days, development of peg; *C*, five days, function of peg in splitting seed coat (*sc*) and withdrawal of cotyledons; *D*, eight days, seedling in which primary growth is completed (*ecl*, epicotyl); *E*, older plant, position of first foliage leaves (*l1* and *l2*) and relative lengths of first (*ino1*) and second (*ino2*) internodes.

The determination of the seedling phase in the life cycle is necessarily somewhat arbitrary. In this case the limit has been placed at about eight days, when the primary structures seem to be completed and most of the stored nutritive material has been utilized so that the young plant is independent and commencing the growth which results in secondary structures (fig. 1*D*). At the end of eight days, then, the plant shows a well branched root system. The primary root is slender, gradually increasing in diameter up to the peg, at

which level the axis enlarges abruptly. This is caused in part by the lateral extension of the peg, and in part by a general enlargement which is maintained through the length of the hypocotyl except for a slight taper below the cotyledons. The hypocotyl is slender and averages about 7.5 cm. in length after elongation is completed. In this variety the surface of the hypocotyl is smooth. The expanded cotyledons are oval, with broad bases, rather thick and green, as is the rest of the plant above the soil. The upper surface of the cotyledon is somewhat hairy but the lower surface is glabrous with prominent veins. The slowly developing epicotyl scarcely shows as yet.

#### PRIMARY STRUCTURES

ROOT TIP.—JANCZEWSKI (17) classified the root tip of *Cucurbita maxima* as the fourth type. He designated this form as characteristic of only the Cucurbitaceae and the Leguminosae. In this type the identification of several separate histogens is impossible; instead there is one common generative zone from which all tissues arise. In a median longitudinal section of the primary root tip in *C. maxima* this generative zone forms a shallow curve across the tip. At the center the meristem may show about seven layers of cambiform cells, the number diminishing toward the margins. Outwardly this generative zone renews the root cap. At the same time the marginal portions give rise to the dermatogen, the dermatogen and root cap layers dividing from each other so as to effect the "stair-step" appearance in their differentiation, such as JANCZEWSKI described for the third type of root. The remainder of the root is derived from cells differentiating inwardly from the central portion of the generative zone. These cells undergo several further divisions which are often irregularly periclinal; then that tissue directly in the center forms the plerome, or stele, and the remaining adjacent cells form the periblem, or cortex.

ROOT AXIS.—From this type of apical meristem differentiation of the various tissues is difficult to follow. In the seedlings studied it is further complicated by the rapid elongation of the primary root and the consequent delayed maturation of the tissues. Approximate delimitation of cortex and stele is soon possible, however, because the cells composing the innermost layer of the cortex continue to



undergo tangential divisions for a longer period of time. Within the stele the first tissue to mature is the protophloem. It consists of four arcs of tissue only two or three cells wide, but tangentially extending ten or more cells. In the center of each band, usually wedged between two larger pericyclic cells, a single cell differentiates through changes in its wall and its contents. This cell with its lightly staining contents is conspicuous among the others in which the cytoplasm is much more dense. This protophloem element, although small in diameter, may be considerably elongated. Soon other similar cells differentiate in the band of protophloem; they also are adjacent to the pericycle or may be one or two cells deeper. Among them are other larger parenchymatous cells of the protophloem.

In contrast with the first formed phloem, consisting only of parenchyma, the metaphloem possesses sieve tubes and companion cells. The former are elongated but are small in diameter with relatively small sieve plates. The companion cells are short and have the nucleus and dense cytoplasm usually characteristic of this type of cell. The greater part of the metaphloem is composed of parenchymatous cells, usually larger in diameter than the sieve tubes, variable in length, but generally several times their width. In addition there are some scattered cells with darkly staining and coarsely reticulate cytoplasm.

Paralleling the maturation of the protophloem, the protoxylem has also begun differentiation. The first elements observable are annular with relatively heavy ring thickenings. There are four of these, one at the apex of each protoxylem point. Usually these elements are soon torn, thus initiating the protoxylem lacunae which may later become conspicuous. At the time that this first protoxylem is maturing, the tetrarch exarch pattern of further differentiation is marked out by the successively larger cells in the four primary xylem arms. The two or three cells next within the oldest protoxylem differentiate as delicate annular or spiral elements. The transition from annular to spiral is irregular. Similarly the next two or three elements show a transition in thickening from a delicate spiral to a heavy scalariform pattern. Because of these intermediate types it is not always possible to distinguish protoxylem from metaxylem, but certainly the next vessels form part of the metaxylem. They are

considerably larger than the outer preceding xylem elements and show closely reticulated thickenings. In addition to the elements just described, a variable number of adjacent cells may undergo similar differentiation.

After maturation of these four strands of primary xylem, a considerable area in which further differentiation is retarded remains in the center. It at first has the appearance of pith. This fact may account for VAN TIEGHEM'S (26) report of pith in the root, since he studied a young section only 1 cm. from the root tip. GÉRARD (13) also noted pith in the root; as he was primarily studying the region of transition, he may have observed a section of the transition at a level in which pith was differentiated, since this tissue descends rather deeply. As RUTLEDGE (24) has noted for *Cucurbita maxima* and as seen in the variety used for this study, ultimately two or more cells at the center mature into conspicuously large, pitted vessels (*cf.* fig. 4A). The parenchymatous cells surrounding these vessels are isodiametric or horizontally elongated, their walls reticulately thickened and often sinuous. These cells may abut directly against the reticulate vessels in the four earlier formed xylem strands, or two or three layers of vertically elongated, thin walled xylem parenchyma cells may intervene.

Between the primary xylem and phloem a band of tissue remains undifferentiated; this tissue constitutes the cambium. Exterior to these primary vascular tissues is the pericycle. Over the phloem it is composed of a single layer of cells, large in diameter and somewhat elongated. Over the protoxylem points it consists of about four layers of cells, smaller in diameter and only slightly elongated.

During maturation of the stele, the cortex has also matured. At first the innermost layer of cortical cells continues to show tangential divisions, adding to the cortex. At the same time the cells in the outermost layers undergo radial divisions. Finally these different divisions cease, and, with maturation of the scalariform elements the innermost layer becomes the endodermis with narrow Casparian strips. The cells are more elongated and somewhat larger in diameter than those of the pericycle. The parenchymatous cells of the cortex are still larger; they are elongated and show conspicuous intercellular

spaces. Completing the axis is the epidermis, composed of elongated tabular cells many of which form root hairs.

ORIGIN OF SECONDARY ROOTS.—Concerning the origin of secondary roots in the Cucurbitaceae, two radically divergent opinions have been expressed. JANCZEWSKI (18) stated that the origin of lateral roots in the Leguminosae and Cucurbitaceae differed from other Phanerogams in that the cortex was involved. In describing the development he declared that the pericycle of the mother root gave rise to the stele of the lateral root, and the endodermis and adjacent cortical layers gave rise to the primary cortex at the surface of which the generative zone developed only later. VAN TIEGHEM and DOULIOT (27), in a reinvestigation of this work, rejected JANCZEWSKI's interpretation. In both *Cucurbita maxima* and *C. pepo* they noted a precise origin of the lateral root from the two pericyclic layers of the mother root, with the endodermis and five or six inner cortical layers forming only a digestive pocket aiding in the outward growth of the young root. The observations made in the present study support the view expressed by JANCZEWSKI.

The primordium of the secondary root originates early in the ontogeny of the primary root, after the differentiation of the proto-phloem but before that of the protoxylem. In several root tips this showed an initiation of the primordium within 1 mm. of the apical meristem, a longitudinal section in particular showing a primordium 0.8 mm. from the generative zone. The first definite indications of development, as traced in transverse sections, are radial divisions in the two or three inner cortical parenchyma layers opposite the protoxylem point (fig. 2A). These divisions are conspicuous in the flatly oval, regularly arranged cells of the cortex. Meanwhile the innermost layer of the cortex continues to exhibit tangential divisions, although in the remainder of the root these are infrequent. At the same time the cells of the pericycle commence dividing rather irregularly, except that the outermost layer, just beneath the tangentially dividing layer of the cortex, also shows tangential divisions. In this way the exact identity of these two layers is soon lost. Longitudinal sections show that the tangential and radial divisions are accompanied by frequent transverse divisions.

Gradually more layers of the cortex become involved in the area

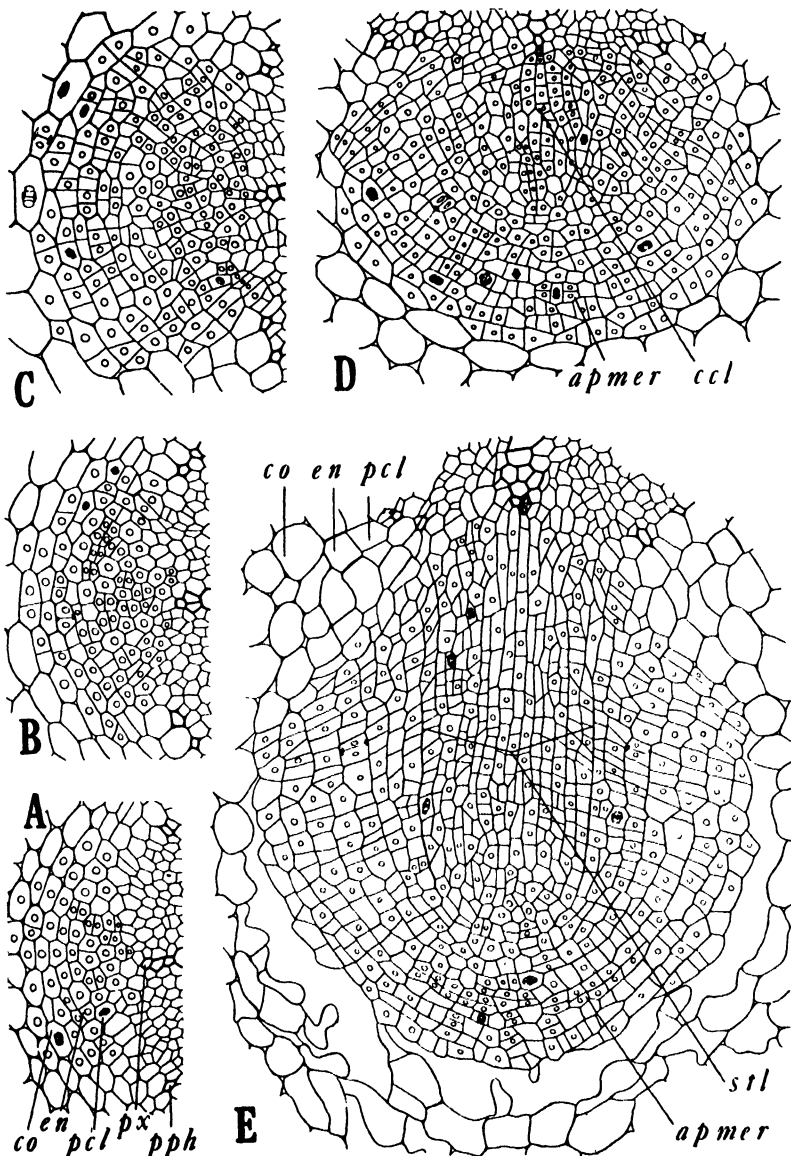


FIG. 2.—Transverse sections from primary root showing origin of secondary root: A, first radial divisions in cortex (*co*), and tangential divisions in endodermal (*en*) and outermost pericyclic (*pcl*) layers, nucleated cells in region of primordium (*pph*, protophloem; *px*, protoxylem); B and C, further divisions in cortex and pericycle; D, continued divisions in cortex and pericycle with formation of central cylinder (*ccl*) and origin of apical meristem (*apmer*); E, secondary root nearly through cortex of primary root; stele (*stl*) and apical meristem established.

showing radial divisions (fig. 2*B*, *C*). The lateral extent of this zone also increases, the base extending toward the phloem on either side. The cortical cells composing it remain meristematic. They show only a single radial division at first but later several divisions occur, those cells earlier involved showing the greater number. The outline of the mother cell remains traceable, also the layered arrangement of the cortex, although the latter is somewhat distorted by the greater activity and growth of the tissue of the pericycle. In this last region successive divisions produce numerous small, radially elongated cells. With this the lateral root is beginning to take form (fig. 2*D*), the central cylinder of narrow elongated cells forming a stele derived from the pericycle, and curved over this a cap of cortical tissue (and possibly of some pericyclic tissue).

Growth of this primordium continues by further divisions and elongation in the central cylinder. The cortical cap maintains its form and position over the stele by means of successive divisions of those cells nearest the phloem of the parent root. Finally, when all but about three layers of the cortex form part of the cap, a generative zone similar to that already described for the primary root tip develops (fig. 2*D*, *E*). It arises by tangential divisions in approximately the second, third, or fourth outermost layers of the cortically derived part of the root primordium. With growth commencing in this apical area and continuing at the base for a short time, the cortical tissue of the primary root soon tears away around the tip of the primordium, thus freeing the young root to continue growth.

#### TRANSITION AND THE PEG

TRANSITION.—The early descriptions by GÉRARD (13) and DANGEARD (7) gave the essential idea of the transition; that is, doubling of the number of xylem strands, similar doubling of the phloem, superimposition of these two tissues with reorientation of the xylem from centripetal, through tangential, to centrifugal maturation, resulting in eight bundles separated by narrow medullary rays. LAMOUNETTE (21) presented a somewhat different account for *Cucurbita maxima*. He traced the division of the four xylem bundles of the root and the formation of four transition bundles alternate in posi-

tion with the former. Of the four bundles, two divided into two each, forming a total of six which completed the transition and con-

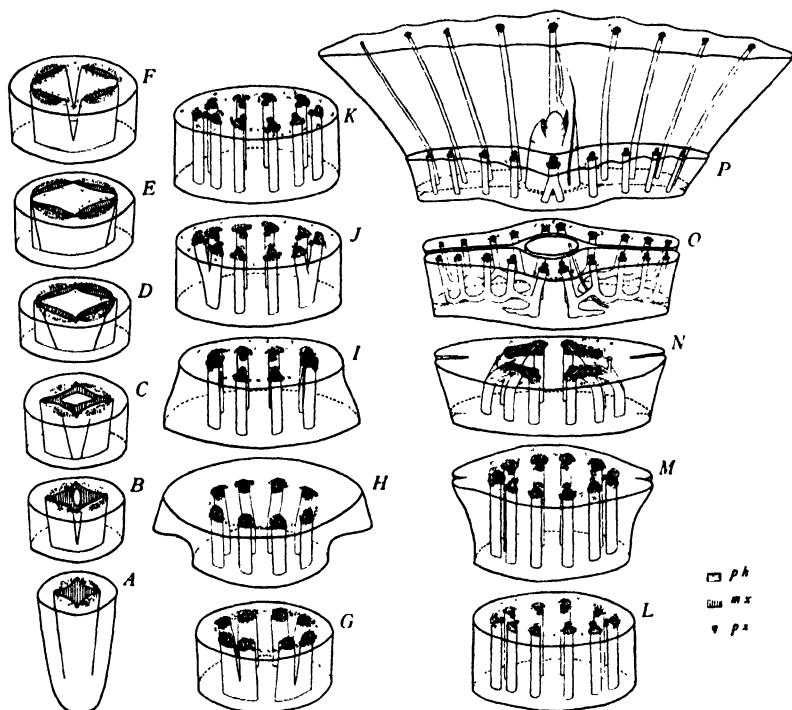


FIG. 3.—Diagram of a seedling, series of transverse sections: *A*, root; *B*, differentiation of pith beginning the transition; *C*, enlargement of pith area with metaxylem in peripheral position; *D*, differentiation of internal phloem; *E*, divergence of each primary xylem strand into two tangential arms; *F*, establishment of dissected siphonostele of four tangential transition bundles; *G*, formation of eight bundles, each with protoxylem still in tangential position; *H*, abrupt enlargement of axis at peg, with protoxylem differentiation becoming endarch; *I*, endarch bundles completing transition, anastomosing of two end pairs of bundles; *J*, end bundles dividing into three to form total of ten bundles which continue through the hypocotyl (*K*, *L*, and *M*); *N*, cotyledonary node, formation of cotyledonary plate by tangential anastomoses, insertion of first foliage leaf trace on dividing end bundle at right; *O*, two median traces to each cotyledon continuing from cotyledonary plate, branching of each trace laterally to establish principal veins in blade of cotyledon; *P*, base of cotyledons, the epicotyl.

tinued through the hypocotyl. Recently RUTLEDGE (24) has supported the two earlier writers. The following agrees with RUTLEDGE except for some details in description and interpretation.

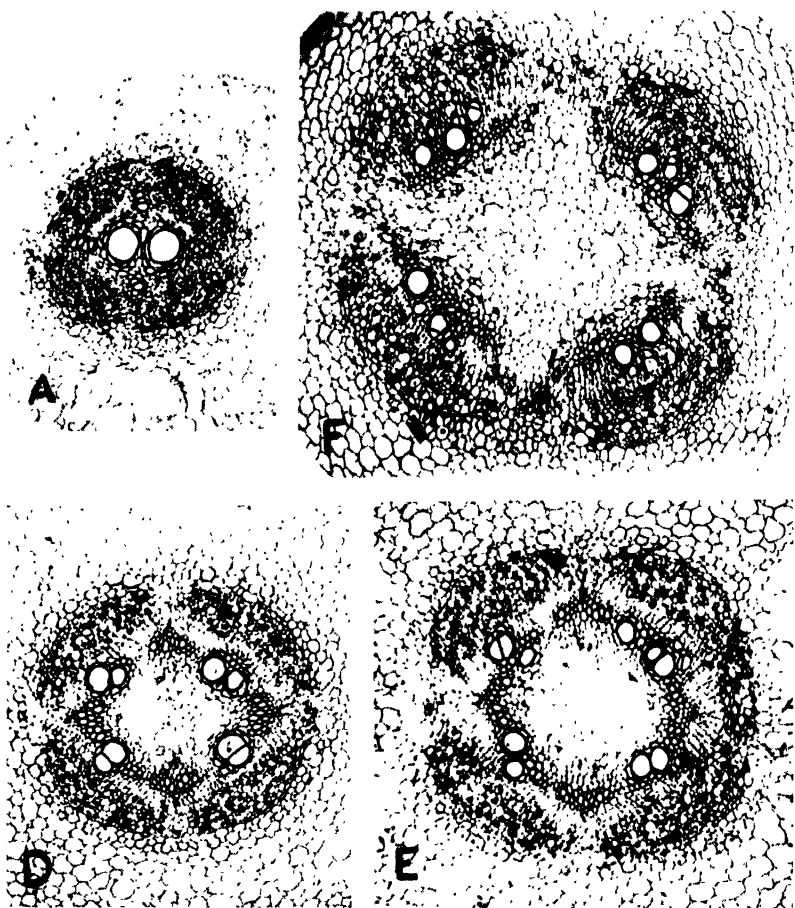


FIG. 4.—Vascular tissues in transition region, series of transverse sections (lettered to correspond with fig. 3): *A*, root just below transition showing differentiation of additional reticulate xylem parenchyma between large metaxylem vessels (*B* and *C* omitted); *D*, central pith and differentiation of internal phloem, identifiable by darkly staining cells within triangular protoxylem strands, metaxylem in peripheral position within external phloem; *E*, divergence of each primary xylem strand into two tangentially extending arms joining the metaxylem; *F*, dissected siphonostele of four transition bundles with tangential band of metaxylem tipped at either end by protoxylem. External phloem masses joined across rays by connective phloem, also phloem along inner face of bundles connected to outer phloem along the rays.

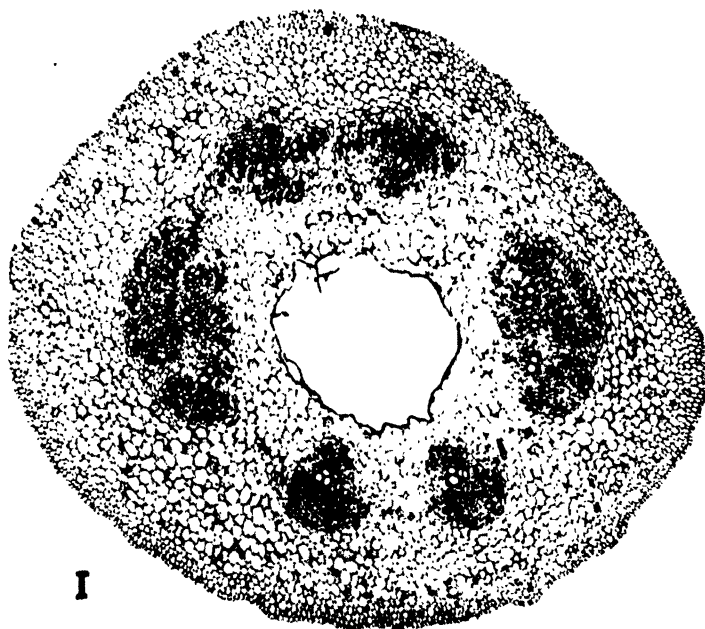
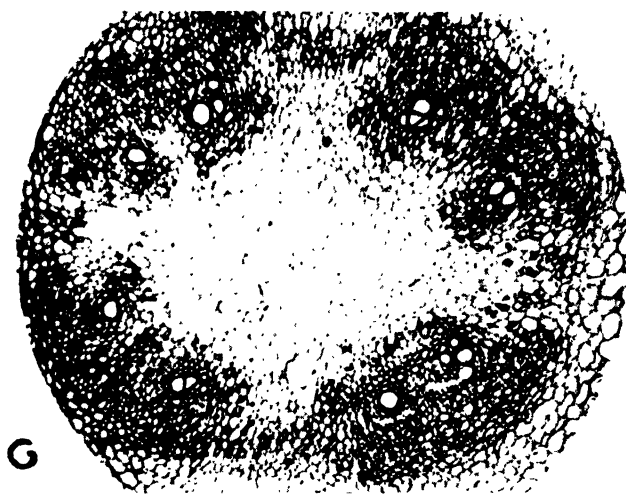


FIG. 5.--Vascular tissues in transition region, transverse sections (lettered to correspond with fig. 3): *G*, eight bundles (one transition bundle just dividing) with phloem differentiating along the new rays, maturation of protoxylem still tangential (*H* omitted); *I*, complete endarch bundles just above peg, also branching of anastomosed end bundle to form the ten vascular bundles of hypocotyl. Differentiation of connective phloem in cortex pericycle, and (less frequently) along the rays.



In *C. maxima* the transition region extends from the exarch protostele in the root somewhat below the peg to the completely endarch dissected siphonostele at a level just above the peg in the stemlike hypocotyl. This involves a portion of the axis over 1 cm. in length in a seedling of about seven days, the exact distance varying with the different specimens. The following description is based on material of that approximate age.

The protostele in proximity to the transition region shows some departure from the structure described as typical of the root (fig. 4A). The reticulately thickened xylem parenchyma cells between the large central pitted vessels have increased in number, especially along a median line extending between two opposite protoxylem points. This has separated the pitted vessels into two groups. At the same time these large vessels gradually increase to four or more in number.

The transition, in tracing the course from root to stem, begins where certain of these median cells differentiate as thin walled, vertically elongated pith parenchyma instead of reticulate xylem parenchyma. A narrow band extending from one primary xylem arm to the opposite and in a line at right angles to the plane of the cotyledons results (fig. 3B). At higher levels this pith increases in width, continuing to the other two lateral arms of the primary xylem (fig. 3C). Concurrent with these changes the pitted vessels by progressively tangential differentiation separate into four groups, which attain positions nearer the periphery of the stele, just within the cambial initials internal to the four phloem groups and alternate with the protoxylem points.

These changes take place gradually, in a spatial relation. Thus about 8 mm. above the wholly rootlike pattern of tissues, the protoxylem and first formed metaxylem still retain the same linear exarch relationship. The inner reticulate tracheae are more numerous, and so broaden the base as to form triangular masses of these primary xylem strands (fig. 4D). Connecting these triangles tangentially, and in this way producing a hollow diamond inclosing the pith, is the remaining and later formed metaxylem with the large pitted vessels. By differentiation the number of these vessels again in-

creases from the approximate four to an approximate eight, two in each tangential mass of metaxylem. These vessels are surrounded by reticulate xylem parenchyma which may abut directly against the reticulate tracheae in the triangular masses, or more usually is separated from them by several layers of thin walled xylem parenchyma, as described for the root. External to the metaxylem are the cambial initials, already active at this stage but with no secondary differentiation. Outside this the primary phloem has maintained its original position and relative arrangement. Similarly the pericycle, cortex, and epidermis show continuity with the lower root.

At successive levels above this, reorientation of tissues occurs more rapidly. Because of slight variations in the rate of this reorientation, all four parts of the stele, even though they undergo the same changes, may not show exactly similar structure at any given level. This is especially true of the upper levels. Continuing up the axis 1 or 2 mm., the pith increases in extent and there is a differentiation of internal phloem (figs. 3*D*, 4*D*). Certain cells, frequently those internal to the xylem triangles but several cells removed, undergo divisions producing groups of smaller cells. Among these are some of the conspicuously darkly staining cells already described in the outer phloem. Others are simple phloem parenchyma. A few cells exhibit end walls which are perforated with numerous and somewhat irregularly arranged simple pits. In this way they are similar to the sieve tubes of the outer primary phloem, but the cells are very much larger, and companion cells have not been noted except as the darkly staining cells take this place.

At this level the xylem is not characteristically exarch as in the root. By the maturation of parenchyma in the inner face of the metaxylem immediately subtending the protoxylem, these first formed reticulate tracheae gradually assume a more tangential position in relation to the annular and spiral elements. At successively higher levels each of the original triangular masses becomes divided into two arms spreading toward the tangential bands of the later formed metaxylem. The latter shows the formation of additional small reticulate tracheae which are arranged at first adjacent to the two larger pitted vessels but which progressively differentiate to a position between the two vessels.

Continuing upward, this process of splitting extends to the protoxylem, resulting in irregularly double rows of annular and spiral elements, so that about 1 mm. higher a hollow square of xylem is formed with protoxylem touching at the corners and the sides formed of metaxylem composed mostly of reticulate tracheae, but with about eight separate pitted vessels (figs. 3*E*, 4*E*). Within this square the internal phloem is distributed along all four faces but remains as scattered groups of cells separated from the lignified elements by several layers of parenchymatous cells, some of which may show divisions initiating cambial activity. The parenchymatous pith is composed of large, somewhat elongated cells, circular in cross section, with conspicuous intercellular spaces. The tissues external to the xylem are arranged much as before, except that the lateral extent of the phloem is greater and the rays opposite the protoxylem points are consequently narrower.

In the next 1-2 mm. higher, the pattern of the primary structures is usually complicated by the development of lateral roots at the protoxylem points. Above these, however, each protoxylem point is completely divided into two parts by the differentiation of an intervening parenchymatous ray. As a result, a dissected siphonostele of four tangential bundles is established at this level (figs. 3*F*, 4*F*). Each of these transition bundles consists of phloem and a tangential band of metaxylem tipped at either end by annular and spiral protoxylem elements. The metaxylem forms a continuous layer of small reticulate tracheae with the two or more larger and later matured pitted vessels occupying a position on the outer face of the tangential band. The internal phloem is continuous across the inner face of these bundles but is interrupted at the four primary rays. It is more closely associated with the xylem than at the lower levels. The outer phloem is still directly continuous with that of the root, having maintained both its original arrangement and position. In addition there is frequently differentiation of phloem across the rays connecting the adjacent areas of external phloem, and differentiation in the parenchyma along the side of the ray connecting the inner and outer phloem masses around each bundle.

The diameter of the axis increases more rapidly toward the level of the peg, the pith is larger, and the bundles are separated by

wider rays. At the same time, and about 1 mm. above the establishment of four bundles, the number of bundles increases to eight (figs. 3G, 5G). This results from the maturation of parenchyma through the center of each tangential bundle. This parenchyma becomes established first in the band of metaxylem interrupting it in the approximate center and so dividing it that each part shows one of the large pitted vessels and other associated tracheae.

Differentiation of the ray through the phloem masses takes place at higher levels, occurring first through the internal phloem and, still higher, through the outer phloem. These eight bundles show characteristics of transition in that the differentiation of the xylem, in passing from the protoxylem to the first formed metaxylem, is still tangential; but the later formation of the large pitted vessels on the outer face of the xylem mass is centrifugal or endarch. The outer phloem retains its relative arrangement except that it now consists of eight masses instead of four. Similarly the internal phloem is present along the inner face of each bundle.

Progressive differentiation from the internal phloem and from the outer phloem in the newly developed parenchymatous ray establishes phloem interconnections along this lateral face just as occurred along the opposite face at a lower level. The internal phloem is separated from the lignified elements by one or two layers of parenchymatous cells which may or may not show cambial activity. Likewise the parenchyma between the lateral phloem and the vascular tissues may show cambial activity. The delimitation of distinct phloem areas is difficult because of this differentiation of phloem across the rays and along the sides of the bundles. Outside the phloem, the parenchymatous pericycle has become irregularly several layered. Next this is the endodermis still traceable as a continuous single layer around the stele, then the parenchymatous cortical tissue, and finally the epidermis which forms root hairs up to the approximate level of the peg.

With the development of the peg the diameter of the axis suddenly increases (fig. 3H). In the lower levels this results from increase in cortical tissue on the one side to form the peg. At a level about midway in the peg, however, the stele may also participate in the general widening, the two median bundles on the side toward

the peg curving abruptly outward and the two adjacent bundles also partly sharing in this outward differentiation. Meanwhile, in a distance of about 1.5 mm., at the upper levels of the peg, reorientation of the primary xylem from tangential to centrifugal or endarch differentiation is completed (figs. 3I, 5I).

Not only does the xylem change in its relative arrangement, but also in the character of the elements composing it. The emphasis on scalariform and reticulate elements characteristic of the root passes to emphasis on spiral or loosely scalariform elements. At the same time there is a gradual decrease in the diameter of the vessels from root to hypocotyl. The phloem shows greater continuity in its character. The outer phloem forms a broad mass capping each bundle, and scattered phloem groups occur across the rays connecting these various outer masses. The internal phloem maintains its position on the inner face of each bundle, but the interconnections with the outer phloem are less frequent. Apparently phloem may also differentiate in the pericycle over the vascular bundles, as the darkly staining cells of the phloem may be found directly adjacent to the endodermis. The endodermis may be traced over the bundles by its small Casparian thickenings, but it is indistinguishable across the rays. At still higher levels it can be identified above the bundles chiefly by its starch containing cells. The remainder of the cortex is composed of large parenchymatous cells with conspicuous intercellular spaces. A cutinized epidermis completes the axis. With the establishment of this dissected siphonostele of endarch bundles the transition is concluded.

The transition in *Cucurbita maxima* is of a fairly simple type. Two features, however, the internal phloem and the peg, add particular interest to it. Differing opinions have been expressed concerning the internal phloem. GÉRARD (13) first described it in the transition region, indicating that this tissue was derived from the outer phloem by inward migration along the rays. LAMOUNETTE (21) denied this, stating that there were no interconnections between the outer and inner phloem, the internal phloem ending blindly below. Further investigation has shown that there is continuity or interconnection of inner and outer phloem, and it is not to be considered that the one developed from the other. Confusion may have arisen

from the fact that the downward extent of the internal phloem seems to vary with the age of the seedling. In a young plant of three or four days it is possible to identify tissue which is internal to the xylem mass of each endarch bundle and which will mature directly into internal phloem. This tissue may be traced to a lower level (approximately fig. 4*G*) where these eight bundles show a tangential arrangement of the xylem, but lower than this it gradually becomes indistinguishable. Progressive downward differentiation takes place so that at about seven days phloem is readily identified at a level where the pith is entirely inclosed by xylem, as described in the transition (fig. 4*D*). Differentiation in the rays seems to correlate with differentiation along the inner face of the bundle. In older stages, therefore, the inner and outer phloem do show interconnections along the rays, but also the internal phloem ends blindly at a still lower level.

PEG.—The peg has presented controversial material in regard to the factors influencing its development, but morphologically the structure is simple. Although the pattern of the transition is already formulated in the embryo, there is no trace of the enlargement which forms the peg. As noted, only on the third day after planting does the peg begin to form as a lateral ridge. Cross sections show that it results from numerous cambial-like divisions in the tissues along a plane extending tangentially across the axis. At lower levels the line of this activity usually involves only the cortex, but at higher levels this plane of divisions may also involve the vascular tissue, which accounts for the abruptly outward course of these bundles.

The broad lower face of the peg is at right angles to the axis, or even at an acute angle; it bears root hairs and often shows traces of reticulately thickened and branched parenchymatous cells which were part of the seed coat to which this surface of the peg was firmly attached during germination. The upper part of the peg gradually merges with the hypocotyl, and shows the smooth epidermis of the latter. CROCKER, KNIGHT, and ROBERTS (6) have pointed out that the position of the peg is determined by external influences. Certainly it does not show a constant morphological relationship to the transition. In some cases the greatest dimension of the peg occurs at the level of the four tangential transition bundles, and in other

specimens this level of maximum extent occurs where there are eight or more completely endarch bundles.

#### HYPOCOTYL

**COURSE OF THE BUNDLES.**— In the transition from root to hypocotyl a change in the form of the axis occurs, from the circular root to the oval hypocotyl. The peg at the base is on one side of this oval. This form continues up to the divergence of the cotyledons, the longer diameter of the oval parallel to the plane of the cotyledons. Of the eight original bundles in the upper levels of the transition, two are at each end of the oval outline and two along each side (fig. 3*H*). Usually just above the peg and often before the vascular bundles becomes endarch, the number of these bundles increases. This is brought about through anastomosing and branching of the eight bundles, most of these changes taking place within a few millimeters above the peg. Ten was the smallest number of bundles found in the hypocotyls observed; twelve is a common number; and as many as sixteen have been counted. This necessarily involves a wide variety of patterns, the simplest and most frequently recurring of which is described. At the upper levels of the transition the two bundles at each end of the oval anastomose to form a single large bundle which continues up the axis for a short distance before it divides into three (figs. 3*I*, 5*I*). These six with the two along each broad face of the oval make ten vascular strands, the basic number. Frequently an additional bundle is formed on each side of the oval by simple branching of one or the other of the original bundles. This pattern of ten or twelve bundles may continue up the axis to the cotyledonary node (fig. 3*I-M*), or any one of these bundles, especially those at the end of the oval, may give rise to one or more additional bundles. Similarly the anastomosing and divergence at the lower levels may vary considerably; the anastomosing may be omitted and branching may be more frequent. These bundles are arranged in a single ring, in contrast to the two rings characteristic of the upper stem of the Cucurbitaceae.

RUTLEDGE (24) described a similar pattern as an exceptional case. He accounts for the increase in number by the formation of additional bundles between the original bundles, frequently between

those on either side of the oval. He does not make it clear as to whether these bundles are independent in origin, or are anastomosed with the adjacent ones. That the latter is the case is shown in material stained and cleared by Gourley's method. The continuity of all parts of the vascular system is evident. It is true that the additional bundles are usually smaller than the others and branching at first involves only phloem, but xylem is later differentiated. Also in certain cases in very young seedlings these additional bundles, after several lateral anastomoses just below the cotyledonary plate, continue for a short time as small strands of phloem which then end blindly.

Contrary to these observations is a statement by DANGEARD (7) that the hypocotyledonary axis in the Cucurbitaceae is later modified by the descent of foliar traces. Since the entire vascular pattern of twelve bundles as described was traced in the procambial strands of an embryo before germination, there is hardly evidence in *Cucurbita maxima* to support this view of DANGEARD.

**DIFFERENTIATION IN THE AXIS.**—The pattern of the hypocotyl formulated in the embryo consists of tissue showing little differentiation. The epidermis is composed of small closely arranged cells. The parenchyma of the cortex and a similar parenchyma of the stele consist of short, thin walled cells filled with stored nutritive matter and showing characteristic intercellular spaces. Surrounded by this fundamental parenchyma, about halfway between the center and the epidermis, are the provascular strands varying in form and size according to their position in the pattern. They are composed of small elongated cells without the dense contents of the parenchyma cells and without intercellular spaces (fig. 6A).

In further differentiation, the first changes occur in the provascular strands. Several elements of the protoxylem mature by the third day. These elements do not differentiate on the inner margin of the procambial tissue, but several cells within it (fig. 6B). They are annular and coarsely spiral elements. Unlike the root they are not necessarily adjacent to one another, but may be separated by xylem parenchyma. These first elements are small in diameter, but the later matured elements are larger and the thickening of their walls



is more closely spiral. In the hypocotyl a spiral-scalariform vessel of elongated segments of relatively large diameter seems to be the

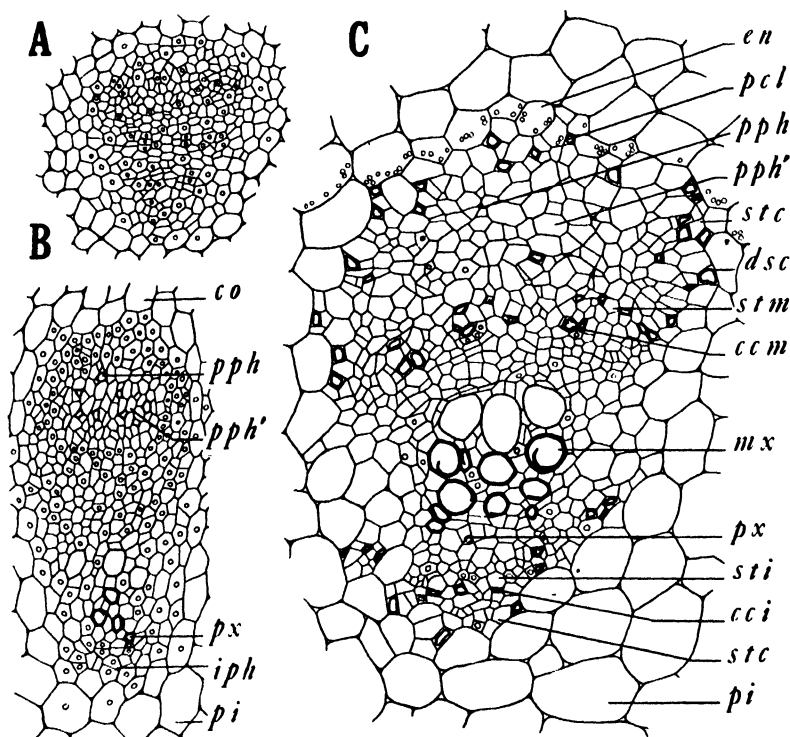


FIG. 6.—Differentiation of vascular bundle in hypocotyl: *A*, provascular strand in embryo just before germination; *B*, vascular bundle of three-day seedling showing outer protophloem with elongated elements (*pph*) and other parenchymatous cells (*pph'*), protoxylem (*px*), and several recently divided cells in undifferentiated internal phloem (*iph*); *C*, vascular bundle of seven-day seedling with primary differentiation nearly completed, showing external phloem with crushed elongated protophloem elements (*pph*) and other protophloem cells (*pph'*) enlarging to become fibers, differentiation of metaphloem with sieve tubes (*stm*) and companion cells (*ccm*), of internal phloem with sieve tubes (*sti*) and companion cells (*cci*), of connective phloem in pericycle (*pcl*) adjacent to endodermis (*en*) and in association with external and internal phloem, chiefly along periphery. Connective phloem conspicuous because of its darkly staining cells (*dsc*); it also shows sieve tubes (*stc*) (*pi*, pith).

end point of primary differentiation, instead of the large, pitted, short segmented trachea such as was found in the root. At the end of primary growth the first protoxylem elements have been torn but

a lacuna is not formed; instead the vessels are closed in by the adjacent enlarging parenchymatous cells.

On the third day the external protophloem is also identifiable (fig. 6*B*). It is similar to that found in the root, with the small, elongated, lightly staining cells interspersed among the larger undifferentiated parenchymatous cells. This protophloem forms a small oval mass of tissue toward the outer face of the bundle. The external metaphloem is much later in differentiation, the sieve tubes and companion cells being first identified in material five days old (fig. 6*C*). In addition the metaphloem shows the darkly staining cells and undifferentiated parenchyma as described for the root. During this later development the small elongated cells of the protophloem are crushed and resorbed. The accompanying parenchymatous cells become enlarged and very elongated. By the eighth day there is scarcely a trace of the crushed cells, and the remaining parenchymatous cells are identifiable as those which later become the lignified phloem fibers characteristic of the hypocotyl (fig. 6*C*).

In the meantime the internal phloem has matured. Since the protoxylem apparently does not form on the inner margin of the provascular strand, one or more layers of procambial tissue remain undifferentiated. There are exceptions to this in some of the smaller additional bundles where the xylem is seemingly formed along the inner margin. The determination of the exact extent of this procambial tissue is impossible. This inner tissue does not show differentiation of protophloem as found in the outer phloem. Instead, during the third and fourth days, cell divisions in these inner layers and adjacent cells increase the extent of this meristematic area (fig. 6*B*). About the fifth day sieve tubes and companion cells are differentiated (fig. 6*C*). Thus the internal phloem corresponds in composition and maturation with the outer metaphloem. Differentiation takes place in those cells toward the pith, and an undifferentiated parenchyma intervenes between the internal phloem and the xylem. It is this zone which later gives rise to the internal cambium but remains inactive during the primary growth.

While the external and internal phloem is maturing, there is also differentiation of phloem strands in non-vascular regions. This unusual distribution was first described by FISCHER (10), who classi-

fied the phloem into several types according to its position: ectocyclic if external to the pericyclic ring of fibers (of the upper stem); entocyclic if within this sclerenchymatous ring; and commissural if connecting these two types and the vascular phloem of the bundles. This classification seems hardly necessary for a study of the hypocotyl. In the first place there is no separation of the ectocyclic and entocyclic systems, but all of these phloem strands form a much branched and interconnected system. So also it is hardly possible to distinguish the commissural phloem. Moreover all this phloem appears structurally similar. On this basis it is simpler to refer to this as connective phloem.

In the primary growth of *Cucurbita maxima* these connective phloem strands appear in the cortical region, in the pericycle, and in the rays around the bundles, although not directly adjacent to the vascular tissue except where the strands occasionally anastomose with either the internal or external phloem. This phloem is abundantly formed in the pericycle. This is especially true over the bundles as noted in the transition region. GÉRARD (13) first described this as interruption of the pericycle by the fiber-phloem bundles. Later RUTLEDGE (24), finding phloem directly adjacent to the endodermis, concluded that the pericycle was lacking here. But since this phloem is definitely external to the protophloem (fig. 6C), it seems more logical to interpret it as differentiation of connective phloem in the pericycle over the bundle.

The connective phloem does not show in the earliest stages of the seedling, but about the fourth or fifth day it begins to develop. This probably accounts for the retarded development of the internal phloem in the transition and similarly along the rays. Certain cells undergo several longitudinal divisions each, resulting in the differentiation of one sieve tube, one or more darkly staining cells, which occupy the position of companion cells, and several parenchymatous cells, all from the one mother cell. In transverse sections of the axis this phloem appears in isolated patches, except where occasional horizontal anastomoses occur. Longitudinally the branched but connected nature of this system is more evident, showing where adjacent mother cells have so differentiated that the sieve tubes are continuous with others vertically or laterally.

Completing the primary maturation of the axis, the other tissues exhibit fewer changes. The epidermis consists of short tabular cells. Stomata are present in the epidermis. Immediately within are several layers of collenchyma. The cortical parenchyma shows large elongated, chlorophyll-containing cells, and the characteristic intercellular spaces have persisted. The endodermis can be traced over the vascular bundles by its conspicuously short cells, which are without Casparian thickenings but contain starch. Across the rays its identity is lost. The pericyclic and pith parenchyma are similar to that of the cortex, except that the inner pith in this variety does not show differentiation of phloem in the primary condition. At the center of the axis a pith lacuna is formed during the later phases of primary growth. This lacuna extends from the upper levels of the transition to just below the cotyledonary node.

Up to this point the description of phloem has followed in general the interpretation given by FISCHER (10); but from certain observations, the question has arisen as to whether there are two types of tissue involved in the phloem. This idea is supported by the work of BRAEMER (3), and a number of his observations parallel those made in *Cucurbita maxima*. The one type of phloem is found only in the vascular bundles, hence can be referred to as fascicular phloem. It is differentiated in both the outer and the inner phloem (fig. 6C'). In it the sieve plates show a callus formation; the perforations of the plate seem small and regular in arrangement. The cell contents of the sieve tube are homogeneous and rather translucent. The companion cells, two or more to the length of one sieve tube, are filled with dense cytoplasm and clearly show a nucleus. The fascicular phloem is derived from procambial tissue and at the most the sieve tube and the accompanying companion cells originate from the one mother cell.

The second type will be referred to as connective phloem since it is this type only which is found in the non-vascular regions and already has been designated by that name. In addition it is associated with both vascular areas of phloem, but chiefly along the periphery of these (fig. 6C). The sieve plates of the connective phloem do not show a callus formation but react to stains as do the adjacent cellulose walls. The perforations in the sieve plates are seemingly larger

and less regular. The sieve tubes may be much shorter than those of the fascicular phloem, and the cell contents show darkly stained, plastid-like bodies scattered through the clear cytoplasm. In this second type of phloem the so-called darkly staining cells are often associated with the sieve tubes as companion cells, but apparently not regularly so. They may be of relatively large size with densely staining, coarsely reticulate cytoplasm and obliterated nuclear outline. In origin this connective phloem appears to be derived from partially differentiated parenchymatous cells. As previously noted, the one mother cell may give rise to a sieve tube, two or more darkly staining cells, and several parenchymatous cells. It is in this connective phloem that BRAEMER found the active constituents of the drugs in the several plants which he studied. Because of this fact, and because it differs so much from the fascicular phloem, he considered it as a segmented lactiferous system. This reinterpretation would eliminate the more unusual aspects of phloem distribution in *C. maxima*. The observations here presented are admittedly limited, however, and suggest only that further work is needed on this subject.

Before concluding the discussion of phloem, reference should be made to the subject of the bicollateral bundle. HÉRAIL (15), an early worker on the problem, formulated two criteria for bicollaterality: (a) the derivation of the internal phloem from the procambial strand, and (b) simultaneous development of the inner and outer phloem. He considered that the Cucurbitaceae had definitely bicollateral bundles. The present description of primary differentiation in the bundle of *C. maxima* is in agreement with this; the procambial tissue gives rise to phloem on the inner face of the bundle and the differentiation of this inner phloem is concurrent with that of the outer metaphloem. Later LAMOUNETTE (21) described the derivation of the internal phloem from the pith only. His work seems to be on an arbitrary basis inasmuch as it is so often impossible to distinguish between procambial tissue of the vascular strand and the pith. Also the consideration of two types of phloem gives further reason for discarding this latter interpretation. On this basis phloem derived from the pith would be only connective phloem. Actually the vascular type is also found in the inner phloem.

BARANETSKY (1), COL (5), and WORSDELL (30) have rejected these two criteria of HÉRAIL and reinterpreted the bundle from a phylogenetic point of view. They have considered it as not bicollateral but composed of two independent bundles, of which the inner may differentiate only phloem or it may later develop the complementary cambium and xylem. VON FABER (28) found that these potentialities need not in the least destroy the unity of the bicollateral bundle. He reaffirmed the concept of its bicollateral nature on the basis of the ontogeny as he found it for the bundle of *Cucurbita pepo* traced from the apical meristem. The present observations on the bundle in the hypocotyl of *C. maxima* as traced from the embryo agree essentially with the work of VON FABER.

Accepting these criteria with emphasis on the ontogeny of the bundle, that of *C. maxima* is bicollateral. On the other hand, the phylogenetic interpretation is equally important, but the limited scope of the present study provides no answer for this aspect of the question.

#### COTYLEDONARY NODE

NODE.—At the apex of the hypocotyl the divergence of the cotyledons forms a complicated first node. Like the lower hypocotyl it may show many variations in its vascular pattern, but again can be reduced to a basic form. Tracing upward from the ten vascular bundles previously described in the hypocotyl, the first change in course occurs in those bundles at either end of the oval outline. About 3–4 mm. below the divergence of the cotyledons (fig. 3*N*) the middle bundle at each end divides into two parts, which separate and pass tangentially upward away from each other and toward the adjacent bundles with which they anastomose. These latter continue the tangential course until each end bundle anastomoses with the respectively adjacent central bundle. This reduces the total number of bundles to four, two in the center of each broad side of the hypocotyl. The upward continuations of these four bundles form the two traces to each of the cotyledons. In the course of this general convergence any intervening additional bundles take part. Additional vascular strands between the central bundles on either side of the oval may add considerable variation to the pattern, but ulti-

mately they also anastomose with the adjacent original bundles. The tangential anastomosing produces the effect of four sloping transverse bundles which might be considered the four parts of a cotyledonary plate (fig. 3*N*). Each of these transverse bundles represents essentially the rejoined parts of a single tangential bundle of the transition.

COTYLEDONS.—The four traces just described continue upward and outward into the broad bases of the cotyledons. Before the divergence of the cotyledons is completed, each trace branches laterally, a single vascular bundle differentiating toward the margin of the cotyledon (fig. 3*O*). The course of this bundle is undulating but in general downward. Occasionally there are direct connections between it and that part of the cotyledonary plate immediately below. Four or more large veins diverge upward from this basal lateral vein. The two median traces also continue into the cotyledon; they may anastomose to form one midvein or they may persist independently but with one subordinate to the other. Thus there is established at the base of the cotyledon a complement of at least nine vascular bundles (fig. 3*O*, *P*) which extend into the blade as the principal veins, diverged slightly and interconnected by a network of smaller veins.

From the hypocotyl into the base of the cotyledons the tissues show a simple continuity. The vascular bundles have a structure similar to those described for the axis. They occupy a position at the center of a rather thick mesophyll which is some fifteen or more cells in depth, is composed of parenchyma like that of the cortex in the axis, and shows the same intercellular spaces and connective phloem. On the adaxial surfaces over the vascular bundles there are areas of collenchyma.

The mesophyll in the blade of the cotyledon has a different appearance. In the areas intervening between the large veins the three adaxial layers are closely arranged palisade tissue, and the remaining twelve layers, more or less, form a spongy parenchyma. All this tissue is photosynthetic, although in the unexpanded cotyledon it functioned as a storage tissue. The compact parenchyma is limited to areas adjacent to the more important veins and scattered connective phloem is still found in this. These principal veins are midway

between the two surfaces, but the smaller veins are found interrupting the third palisade layer nearer the adaxial surface. Internal phloem is present in the veins even when only one xylem element is differentiated. The upper epidermis is composed of somewhat larger cells than the lower and it may bear multicellular hairs, but the lower surface is smooth. Stomata appear in both surfaces.

EPICOTYL.—At germination the epicotyl consists of a small growing point overarched by the primordium of the first leaf. In eight days the primordia of six leaves may have differentiated, but the whole structure remains inconspicuous and hidden at the base of and between the two cotyledons (fig. 1D). In about two weeks the first leaf has expanded. It is alternate with the cotyledons and is usually found immediately above these, resulting in a short first internode while the internodes next following are much longer (fig. 1E). Observation of the embryo before germination reveals that the median trace to the first leaf is already identifiable as a procambial strand although the remainder of the tissue in the epicotyl is undifferentiated. This trace shows correspondingly early differentiation of the xylem elements. Possibly the formation of the short first internode is correlated with this precocious maturation.

Material collected at the end of five weeks, stained, and cleared by Gourley's method showed the vascular pattern of the lower nodes and internodes and the relationship of the epicotyl to the lower part of the plant. Contrary to DANGEARD (7), the trace to the first leaf may have the lowest insertion, which is on one or (with branching) on both divergences of the first dividing end bundle of the cotyledonary node (fig. 3N). The other bundles of the stem are later differentiated against the lower and more lateral portions of the transverse bundles which form the cotyledonary plate. This produces a leaf gap opposite each cotyledon. The number of bundles and the pattern formed in the first internode are irregular in the several specimens examined. It is only in the second and third internodes that the characteristic two rings of bundles are established. MANTEUFFEL (22) has noted a similar variation in the lower nodes of *Cucurbita pepo*. In general his description of the course of bundles in the upper nodes and the divergence of the leaf traces is similar to that found in *C. maxima*.



### Summary

1. The root tip of *Cucurbita maxima* possesses a single histogen from which all the primary root tissues arise.

2. The primary root is exarch, tetrarch. Differentiation of the large central metaxylem vessels is retarded; pith is not present.

3. The primordium of a secondary root is formed from the cortex, including the endodermis, as well as the pericycle of the primary root.

4. The transition extends from approximately 1 cm. below the peg to just above it. At the lowest level pith differentiates in the center and the metaxylem takes a peripheral position just within the phloem. Each primary xylem strand diverges into two arms extending laterally and joining the metaxylem. These arms separate, resulting in a siphonostele of four tangential transition bundles. These divide into two parts each, forming a total of eight bundles which become endarch.

5. Of these eight bundles usually two pairs anastomose, then divide into three, producing a total of ten bundles which continue through the hypocotyl. Additional bundles may arise.

6. The bundle is considered bicollateral on the basis of ontogeny; it shows a differentiation of internal phloem from the procambial tissue at the same time that the external metaphloem differentiates. (The study of a single species allows no interpretation on the basis of phylogeny.)

7. A suggestion is made concerning the differentiation of two types of phloem, the one called fascicular phloem and the other called connective phloem. Differences in origin, structure, and distribution of the two types are described.

8. In the cotyledonary node tangential anastomoses produce a cotyledonary plate of four parts. Continuations from these form two traces to each cotyledon. Before the cotyledon diverges completely, each trace branches laterally to form a basal vein from which arise four or more bundles which are the principal veins in the blade of the cotyledon.

9. The bundles of the epicotyl differentiate against the parts of the cotyledonary plate. The epicotyl is retarded in its development

except for the median trace to the first foliage leaf. The early differentiation of this trace may account for the characteristic short first internode.

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# LONGEVITY OF SEED AND ESTABLISHMENT OF SEEDLINGS IN SPECIES OF *POPULUS*

E. H. MOSS

## Introduction

Reproduction of poplars under natural conditions in Alberta, Canada, is ordinarily by root suckers and rarely by means of seeds, in spite of abundant seed production in most years and rapid development of seedlings when moisture is supplied to fresh seeds. The present investigation was begun with a view to a clearer understanding of the conditions under which poplars may become established from seed. As set forth in an earlier paper (2), growth of poplars from seeds seemed obviously to depend not only upon climatic and edaphic conditions favorable to the production and establishment of seedlings, but also upon the transfer of seeds in a viable condition. Thus the question naturally arose as to how long the seed will remain viable under various environmental conditions, more especially in the rather dry atmosphere of Alberta. The writer began his investigation without knowledge of the results already published by Japanese workers (3), and with the expectation that subjection of poplar seeds to arid atmospheric conditions would cause rapid loss of vitality.

It has long been known that seeds of *salices*, while capable of vigorous germination as soon as shed, have only a short period of germinability under ordinary conditions. In comparatively recent years it has been demonstrated that the life of these seeds can be greatly prolonged by appropriate drying at maturity, followed by storage over hygroscopic substances at low temperatures. Certain earlier reports on the prolongation of viability of *salices* have been summarized by NAKAJIMA (3), who obtained striking results with two species of *Salix*, whose seeds under ordinary conditions retained their vitality for only about one week but under appropriate treatment remained viable for as long as one year. In two species of *Populus* investigated by FAUST (1), longevity is said to be greatly

extended when the seeds are first dried for about five days and then stored in closed bottles at a constant temperature of 5° C. Seeds of *Populus grandidentata* thus treated showed 44 per cent viability after forty-five months; seeds not subjected to any special drying or storage conditions lost their power to germinate after a few weeks. Whereas NAKAJIMA demonstrated the effect of preliminary refrigeration in an icebox followed by storage over hygroscopic substances, FAUST showed the importance of preliminary air-drying and of subsequent storage in closed vessels and at low temperatures. I have been particularly concerned with demonstrating the correlation between various degrees of humidity and longevity and also the combined effect of low humidity and low temperature during storage. In many respects my experimentation and results are similar to those of NAKAJIMA and FAUST, although with one exception the species employed are different.

### Experiments on longevity

#### SPECIES INVESTIGATED AND PHENOLOGICAL DATA

Most of the experiments were with seeds of the aspen, *Populus tremuloides* Michx., the balsam poplar, *P. balsamifera* L., and one of the Russian poplars, *P. petrowskyana* Schneid. A few tests were made on the western cottonwood, *P. sargentii* Dode, and on five species of *Salix*. In all of the experiments, material gathered at Edmonton, Alberta, was used. Since 1931, when the investigation began, the aspen has produced heavy crops of seed in three of the seven years and comparatively little or no seed in the other years. The date of maximum seed shedding for aspen varied greatly from year to year, ranging from May 19 (1931) to June 10 (1935). The flowering dates of aspen for the past twelve years have usually fallen in the third or fourth weeks of April, but range from April 11 (1930) to May 4 (1935), the criterion of flowering being the fully elongated male catkin. Approximately six weeks elapse between anthesis and seed shedding. Records of seed shedding by the balsam and Russian poplars have been kept for the past six years, in all of which an abundance of seed was formed by these species. The balsam poplar begins seed dispersal about two days prior to the Russian poplar and likewise reaches the height of shedding about two

days earlier. Although usually in the third week of June, dispersal has ranged from approximately June 4 (1934) to July 2 (1935). For both balsam and Russian poplars the time between anthesis and maturation of seed has been between forty and forty-seven days. Records of three years for the western cottonwood indicate that this species sheds its seeds at Edmonton during the latter part of July or the early part of August.

#### EXPERIMENTAL METHODS

Collections were made when the wool and seeds were emerging from the capsules, only the larger and plumper seeds being used. In the earlier experiments a portion of each collection was placed in a stoppered glass vial at once, another portion was exposed to the atmosphere of the laboratory, and a third portion was placed in a calcium chloride desiccator. After it became apparent that desiccation had the effect, not of reducing but of extending the period of viability, freshly collected seeds were usually allowed to dry for two or three days, exposed to the atmosphere of a dry room at a temperature of approximately  $21^{\circ}$  C., before being placed under any special storage conditions. This air-drying treatment was somewhat similar to that recently reported by FAUST to make for greatest longevity of the seeds, differing mainly in being a day or two shorter and carried out at a temperature usually about  $3^{\circ}$  lower.

Following air-drying, the seeds were subjected to various storage conditions. A portion of each collection was kept in an open dish, out of direct sunlight, in a room of fairly uniform temperature and humidity. Portions of certain of the collections were stored: in corked bottles at room temperature and at  $-5^{\circ}$  C.; over calcium chloride at room temperature and at  $-5^{\circ}$  C.; over sulphuric acid mixtures designed to provide definite humidities. Details of these various storage conditions are given later.

Seeds were tested for germinability by placing on moist filter paper in petri dishes at room temperature, twenty to forty seeds being used for each test. The usual practice was to record percentage and vigor of germination at the end of two, three, and four day periods. Germination was recorded as follows: (a) "vigorous," when a sturdy hypocotyl bore the cotyledons well aloft within the first two

days; (b) "fair," when the same degree of development required two to three days; (c) "weak," when eventually the hypocotyl elevated the cotyledons well above the filter paper; (d) "no germination," when there was no evidence whatever of life, also when the emerging cotyledons and hypocotyl failed to develop to the degree described as weak. It was found, especially under certain storage treatments, that the seeds retained a sluggish vitality long after power to form normal seedlings was lost.

### RESULTS AND DISCUSSION

LONGEVITY UNDER ROOM CONDITIONS.—Room temperature varied from about 18° to 27° C., but nearly always was close to 21° C. The relative humidity was not determined except during one brief period, but available information points to this having been between 40 and 50 per cent during most of the experiments. For the aspen, almost 100 per cent vigorous germination was shown for the first four weeks, after which there was a gradual falling off to 45 per cent vigorous germination at the end of eight weeks. Thereafter viability was lost quickly, dropping to 10 per cent fair and 30 per cent weak germination in twelve weeks, with complete loss of vitality within fifteen weeks. By way of comparison, it may be noted that FAUST reports 100 per cent viability at the end of five weeks for aspen seeds stored in open dishes.

LONGEVITY OF DIFFERENT SALICES.—The balsam poplar closely resembles the aspen, while the Russian poplar has a somewhat longer period of viability under the same conditions. The Russian poplar, stored in open dishes in the laboratory, gave 75 per cent vigorous germination at the end of eight weeks, and still showed some vitality, although no true germination, at the end of fifteen weeks. The results for western cottonwood are less reliable, but indicate a period of viability similar to that of the aspen and balsam poplar.

In general, willows apparently have shorter lived seeds than poplars. At the same time, different species of *Salix* probably show wide differences in seed longevity. These conclusions are indicated in NAKAJIMA's review of literature and experimental work, and are given some support by my experiments. *Salix bebbiana* Sarg. showed practically 100 per cent vigorous germination during the first ten

days under room conditions, after which there was a gradual drop to 50 per cent vigorous germination at the end of six weeks, and thereafter a rapid loss in vitality. Thus this willow appears to have a somewhat shorter period of longevity than any of the poplars under experimentation. Four additional species of *Salix* were tested, *S. candida* Flügge, *S. petiolaris* J. E. Smith, *S. planifolia* Pursh., and *S. discolor* Muhl., and all were found to have shorter periods of seed longevity than *S. bebbiana*. Since these species were tested during one season only, and since the last two species gave only 50 per cent germination even when the seeds were still fresh, too much reliance should not be placed upon the following details. In contrast with *S. bebbiana*, which exhibited a sudden falling-off after six weeks, *S. candida* and *S. petiolaris* showed a similar loss in vitality in about three weeks, *S. planifolia* and *S. discolor* in somewhat less than two weeks.

Seeds of the poplar species and of *Salix bebbiana* stored in stoppered bottles remained viable for considerably longer periods than those kept in open bottles or trays. This difference in longevity is probably due in the main to prevention of humidity (vapor pressure) fluctuations in the closed vessels.

**HUMIDITY AND LONGEVITY.**—Samples of the earlier collections were stored in a calcium chloride desiccator at room temperature. The calcium chloride had been in use for some time in the desiccator and no doubt was hydrated, but the degree of hydration was not determined. Seeds thus stored remained viable much longer than those exposed to the atmosphere of the room.

In order to gain a clearer understanding of the relationship between humidity and longevity, seeds were stored over sulphuric acid solutions designed to provide different degrees of humidity, ranging from extremely dry to very humid. Data on the requisite strength of sulphuric acid required to give definite humidities were obtained from a table published by WILSON (4). In this table, percentages of sulphuric acid are given for relative humidities of 10, 25, 35, 50, 65, 75, and 90 per cent at 0° C. and also at 25° C. By interpolation the writer arrived at percentages of the acid required for the same relative humidities at 20° C., which is close to the mean temperature at which the experiments were run. Then, using the curve for 20° C.,



approximate percentages of sulphuric acid were found for two additional humidities, 83 and 15 per cent; also, by extrapolation of the curve, approximate percentages of the acid were determined for extremely low relative humidities: 5.0, 2.0, 0.5, and zero, the last condition being at least closely approached by use of the concentrated (c.p.) acid. The acid solutions were introduced into wide-mouthed bottles fitted with rubber stoppers, the seeds being held in small cotton bags suspended always at a distance of approximately 3 cm. above the acid. The same volume of acid solution was introduced

TABLE 1

PERCENTAGE OF VIGOROUS GERMINATION OF RUSSIAN AND  
BALSAM POPLARS, AFTER STORAGE AT DIFFERENT  
RELATIVE HUMIDITIES

PERCENTAGE		TIME OF STORAGE IN DAYS					
H <sub>2</sub> SO <sub>4</sub>	RELATIVE HUMIDITY	15	22	39	56	80	127
64.5.....	10	90	80	90	90	70	25
55.5.....	25	85	95	85	75	30	0
50.7.....	35	85	80	80	55	5	0
43.1.....	50	85	5	0	0		
35.8.....	65	0	0				
30.2.....	75	0	0				
64.5.....	10	80	90	100	90	60	0
55.5.....	25	75	100	100	65	0	0
50.7.....	35	75	75	90	45	0	0
43.1.....	50	45	0	0	0		
35.8.....	65	0	0				
30.2.....	75	0	0				

into each bottle, the proportion of liquid to gas volume in the bottle being about one to two.

In the first set of experiments, those of 1935, seeds of balsam and Russian poplars were tested under the humidity conditions shown in table 1. In the second set of experiments, those of 1936, aspen seeds were used and subjected to the more extensive range of humidities shown in table 2. To simplify the presentation, percentages of "vigorous" germination only are included in the tables, percentages of "fair" and "weak" germination being omitted. A certain amount of vitality was regularly exhibited by seeds for some time after power to

germinate, in the sense already defined, had been lost. For example, aspen seeds, stored at 10 per cent relative humidity (table 2), displayed some vitality even after 326 days, but no true germination at that time; although the hypocotyl of a few of these seeds emerged slowly for a short distance, all growth then ceased.

Although the tables show the same general trend for all three species, viability of the aspen is apparently lost at essentially the same rate under the different humidities from 10 to 50 per cent, while viability of the Russian and balsam poplars is retained much

TABLE 2  
PERCENTAGE OF VIGOROUS GERMINATION OF ASPEN AFTER  
STORAGE AT DIFFERENT RELATIVE HUMIDITIES

PERCENTAGE		TIME OF STORAGE IN DAYS									
H <sub>2</sub> SO <sub>4</sub>	RELATIVE HUMIDITY	2	11	16	25	44	53	65	73	105	326
conc.	0.0	90	85	75	15	0	0	.....	.....	.....	.....
95.0	0.5	100	90	75	20	0	0	.....	.....	.....	.....
85.0	2	100	95	80	30	0	0	.....	.....	.....	.....
75.0	5	100	95	80	50	5	15	0	0	0	.....
64.5	10	100	100	95	90	25	25	10	10	20	0
60.0	15	100	100	100	95	55	50	25	15	10	0
55.5	25	100	100	100	95	55	30	30	20	15	0
50.7	35	100	100	95	95	45	45	35	20	10	0
43.1	50	100	100	95	90	40	30	10	15	10	0
35.8	65	100	100	85	80	5	0	0	.....	.....	.....
30.2	75	100	95	90	65	0	0	.....	.....	.....	.....
27.0	83	95	90	80	10	0	0	.....	.....	.....	.....
18.4	90	40	0	0	.....	.....	.....	.....	.....	.....	.....

longer at 10 per cent than at the higher humidities. The data for aspen are regarded as unreliable in respect to the details already noted, however, and for the following reason. The aspen seeds were collected over a period of four days, and hence different portions had received different degrees of air-drying before being more or less thoroughly mixed together for the experiments; this probably accounts in large part for the somewhat erratic nature of the results for critical stages in the longevity of the seeds. Unfortunately tests were not made between 105 and 326 days, but at the latter time only the seeds stored at 10 per cent relative humidity still retained any

degree of vitality; this indicates a mode of behavior for aspen like that of the other two poplars. Inconsistent aspects of the results shown in table 1 are due to a certain portion of the seeds being non-viable from the beginning.

From these experiments the following conclusions are drawn: (a) Russian poplar has a greater seed longevity than balsam poplar and aspen, a conclusion already reached from earlier experiments. (b) There is a striking correlation between longevity and humidity of the atmosphere during storage. Leaving out of consideration extremely dry conditions (below 10 per cent relative humidity), the drier the atmosphere, the longer the life of the seed, and vice versa. (c) Russian poplar, which under room conditions decreases rapidly in viability after eight weeks, shows a corresponding decline only after eleven weeks when stored in an atmosphere of 10 per cent relative humidity. Balsam poplar and aspen behave in similar fashion. (d) Loss of viability is extremely rapid when seeds are exposed to the more humid atmospheric conditions. To cite an extreme case, aspen seeds after only two days' exposure to a relative humidity of 90 per cent had dropped to 40 per cent vigorous germination, and after eleven days had lost practically all vitality. (e) There is a wide difference between relative humidities of 65 and 50 per cent in their effects upon longevity, as shown by the tabular data. This conclusion has an important bearing upon questions of longevity under natural conditions. (f) The optimum relative humidity for longevity appears to be approximately 10 per cent, for in still drier atmospheres longevity is markedly reduced (table 2). There is a possibility, however, that this reduction in longevity of seeds stored over the stronger acid solutions is due, not to excessive drying, but rather to the toxic action of sulphur trioxide. That there was an appreciable amount of this gas in the atmosphere, at least over concentrated and over 90 per cent sulphuric acid, was evidenced by the turning brown of the cotton bags within forty-four days; by this time the seeds in the bags had lost their vitality.

LONGEVITY AT A LOW TEMPERATURE.—Seeds were placed in a glass vial provided with a stopper and maintained in an electric refrigerator at  $-5^{\circ}\text{C}$ . At monthly intervals samples were removed for germination tests. No special care was taken to effect gradual

thawing out of the seeds. Marked extension of longevity was found. Balsam poplar gave 90 per cent vigorous germination after sixteen weeks, and 15 per cent fair germination after thirty weeks. Russian poplar showed 90 per cent vigorous germination after thirty-three weeks and 57 per cent vigorous germination after fifty-two weeks, following which vitality was quickly lost.

The effect of storing seeds in a dry atmosphere at a low temperature was determined. Seeds of Russian poplar were put into a small cotton bag and kept in a large glass vial which was half filled with calcium chloride, the bag being separated from the salt by a thin layer of glass wool. The vial was maintained in the electric refrigerator at  $-5^{\circ}\text{C}$ . Remarkable prolongation of longevity was thereby effected. Even after two years seeds thus treated gave 70 per cent vigorous germination.

These results, considered in conjunction with those obtained by NAKAJIMA and FAUST, indicate that seeds of many salices could be maintained so as to have a high percentage of vigorous germination for several years if subjected to appropriate drying and storage conditions.

LONGEVITY UNDER NATURAL CONDITIONS.—In order to determine the effects of certain environmental factors upon longevity, the following experiments were performed.

Seeds were stored on the sill of an east window in such a way as to expose them to fluctuations in atmospheric temperature and humidity without becoming wetted by rain. During the two months, June and July, through the greater part of which this experiment extended, official figures show that the daily relative humidity readings for 5:40 A.M. averaged 77 per cent and for 5:40 P.M. 51 per cent. The mean relative humidity for the period was almost certainly somewhat lower than the average (64 per cent) of these morning and late afternoon records, and probably was well below 60 per cent. Longevity was found to be little more than half that for seeds stored on the laboratory table. Russian poplar, for example, gave a high percentage of vigorous germination for only about four weeks.

To gain some idea of the effect on longevity of alternate wetting and drying, fresh seeds of Russian poplar were dried for two days and then immersed in distilled water for fifteen minutes, after which the

water was drained off and the seeds spread out carefully in a tray to dry. This treatment was repeated at intervals of two days for a twelve day period. In a parallel experiment the period of each soaking was sixty minutes. Germination tests showed that viability of the seeds was soon lost, especially when the period of each wetting was sixty minutes. At the end of twelve days, both lots still germinated fairly well, but thereafter viability was gradually lost. At seventeen days the first lot showed 40 per cent and the second lot 10 per cent fair germination. Five days later there was no genuine germination, although many of the seeds exhibited a sluggish vitality.

From these experiments and those on relative humidity, it may be concluded that longevity of poplar seeds under natural conditions in central Alberta is rather short, usually between two and four weeks, but varying considerably with the species, with the season, and with local environmental conditions. In more humid climates, seed longevity of poplar species would no doubt be considerably shorter than in Alberta.

### **Establishment of seedlings**

#### **OBSERVATIONS AND EXPERIMENTS**

A number of experiments have been performed in the hope of discovering those climatic conditions in central Alberta favoring the establishment of poplar seedlings. Earlier field observations in the region had shown no evidence of poplars having grown beyond the very early seedling stage under natural conditions. Not infrequently, during a period of damp weather in June, great numbers of poplar seedlings have appeared in gardens and other places, usually in shaded locations, but these have not been observed to live beyond a few days at most.

**GROWTH OF SEEDLINGS IN LABORATORY.**—Earlier attempts at growing poplars from seeds in the garden were unsuccessful even though the seed-bed was protected from direct sunlight and watered regularly. At the same time plants were easily grown indoors, especially when the pots or flats were covered for a few days with glass. It was found that the glass might be removed after three days, without loss of seedlings, even when these were exposed to strong sun-

light from a west window. When glass covers were not used, care had to be taken to add water to the soil frequently, also to avoid exposure to direct sunlight and strong air currents during the first few days. Best results indoors were obtained when the seeds were sown on the surface of sand covered soil, although sowings on compost soil without a sand covering were also successful when proper care was taken in watering.

In short, development and survival of poplar seedlings was found to depend upon the surface layer of the soil being maintained in a moist condition. This cannot easily be realized in a dry atmosphere, except by a close covering, such as is afforded by a glass plate over a flower pot. Success depended also upon watering being done so as to avoid flooding, for the young plants collapsed, often with failure to recover, in a film of water on the soil surface.

MORPHOLOGY OF SEEDLINGS.—The behavior just described appears to be due to an unusual morphological feature of these seedlings, a feature described by YANCHEVSKY (5). At the junction of the hypocotyl and root there arises a brush of long delicate hairs whose distal ends adhere to particles in the surface layer of the soil. While the hypocotyl and cotyledons develop rapidly, the root makes very slow growth for several days, the absorptive function being largely performed at first by the brush of hairs. YANCHEVSKY regards the brush of hairs as a successful adaptation because, with a small amount of stored food material, it gives the largest absorbing surface, the greater part of the reserve food being available for the early development of photosynthetic organs. In other respects, however, the brush of hairs cannot be considered adaptive, for seedlings may succumb when the root extends slowly into the soil and when dependence for water supply is chiefly upon this brush of hairs. When the surface layer of the soil becomes dry, the seedling is unable to obtain moisture. It may be too that drying, especially when a soil crust forms, causes mechanical injury to the hairs and hypocotyl, for YANCHEVSKY reports that sand particles adhere so tenaciously to the hairs that it is impossible to separate them without injuring the seedling. Furthermore, flooding the surface of the soil with water may also have the effect of wrenching the seedling from its anchorage and doing irreparable damage.

SEEDLINGS UNDER NATURAL CONDITIONS. —Pots with young seedlings (four to six days in age) of Russian and balsam poplars were transferred from the laboratory to the garden and sunk almost to their full depth in the soil. Different pots were located as follows: (a) in the open, glass covered; (b) in the open, uncovered; (c) in the open, uncovered but protected from direct sunlight; (d) in deep shade, glass covered; (e) in deep shade, uncovered. In one series water was supplied so as to prevent the surface layer of the soil from becoming dry at any time. This involved frequent watering of those fully exposed. In a parallel series, water was supplied less regularly, with the result that the soil in the unprotected pots soon became dry at the surface. The experiments extended over a period of one month, during part of which the heat was extreme.

In the first series there was practically no mortality, except in (a) where all of the plants had collapsed within two days. Here the damage was apparently due in part to the extreme heat below the glass and in part to water drops condensed on the glass, falling upon the plants. Some of the seedlings made poor growth: those in extreme shade apparently because of insufficient light; those in the open because of strong light, extreme heat, and drying winds. Comparatively little damage was done by rain. The most surprising result was the survival of the seedlings in (b), demonstrating that young poplar plants are capable of growth when exposed to extremely hot and dry atmospheric conditions provided the surface layer of the soil continues moist.

In the second series, seedlings in the uncovered pots collapsed sooner or later, the time of collapse coinciding closely with the drying out of the soil in the upper part of the pot.

Here again root development of the seedlings is of interest. A limited number of observations indicate that growth of the root system takes place slowly during the first month. For example, Russian poplars, grown indoors but in fairly strong light, were found at the end of one month to have tap roots averaging only 2.5 cm. in length. It may be added that subsequent development of the root system is much more rapid. By autumn the root systems of the seedlings grown in pots and flats were very extensively branched. By that time too, the plants were capable of enduring rigorous conditions.

### Discussion

Establishment of poplar seedlings in the continental climate of central Alberta appears to depend upon the surface layer of the soil being maintained in a moist condition during at least the first week of their growth. Moisture must be available in the upper layer of soil because of the slow growth into the soil of the seedling root, and because absorption at that time is largely dependent upon a brush of delicate hairs near the soil surface. Only rarely in Alberta is the soil surface continuously moist during any considerable period in June and July; although June is generally the wettest month of the year, periods of wet weather are ordinarily short and alternate with periods of low humidity and drying winds. It is conceivable, however, that in the exceptional season—continuous wet weather over a period of one to three weeks—poplar plants may become established from seed.

Finally, an interesting contrast may be noted. The conditions favorable for the establishment of seedlings would be unfavorable towards maintenance of viability of seed. Conversely, the conditions unfavorable for the establishment of seedlings would favor maintenance of seed viability.

### Summary

1. The more important results were obtained with the aspen (*Populus tremuloides*), the balsam poplar (*P. balsamifera*), and the Russian poplar (*P. petrowskyana*).

2. Aspen seeds stored in open dishes in the laboratory usually gave 100 per cent vigorous germination at the end of four weeks and 45 per cent vigorous germination at the end of eight weeks, after which viability was quickly lost.

3. Balsam poplar resembles the aspen in longevity of seed, while the Russian poplar has a somewhat longer period of viability under the same conditions.

4. There is a striking correlation between longevity and the humidity of the atmosphere during storage. The optimum relative humidity for longevity appears to be approximately 10 per cent. With increase in relative humidity there is a reduction in longevity.



Loss of viability is extremely rapid when seeds are exposed to the more humid atmospheric conditions.

5. Seeds stored at  $-5^{\circ}$  C. exhibit marked extension of longevity. Seeds stored over calcium chloride at  $-5^{\circ}$  C. show remarkable prolongation of life. Even after two years seeds of Russian poplar thus treated gave 70 per cent vigorous germination.

6. It is concluded that longevity of poplar seeds under natural conditions in central Alberta is from two to four weeks, varying with the species, the season, and local environmental conditions.

7. There is considerable evidence that establishment of poplar seedlings under natural conditions occurs only when the surface layer of the soil is continuously moist during at least the first week of their growth. This is due to the slow growth into the soil of the primary root and also to absorption being largely dependent upon a brush of delicate hairs near the soil surface.

The writer is indebted to Dr. A. W. HENRY for the privilege of using constant temperature refrigerators in the Department of Field Crops, University of Alberta. He is also indebted to the Director of Forestry and to Dr. C. HEIMBURGER, Forest Service of Canada, for an English translation of YANCHEVSKY's paper.

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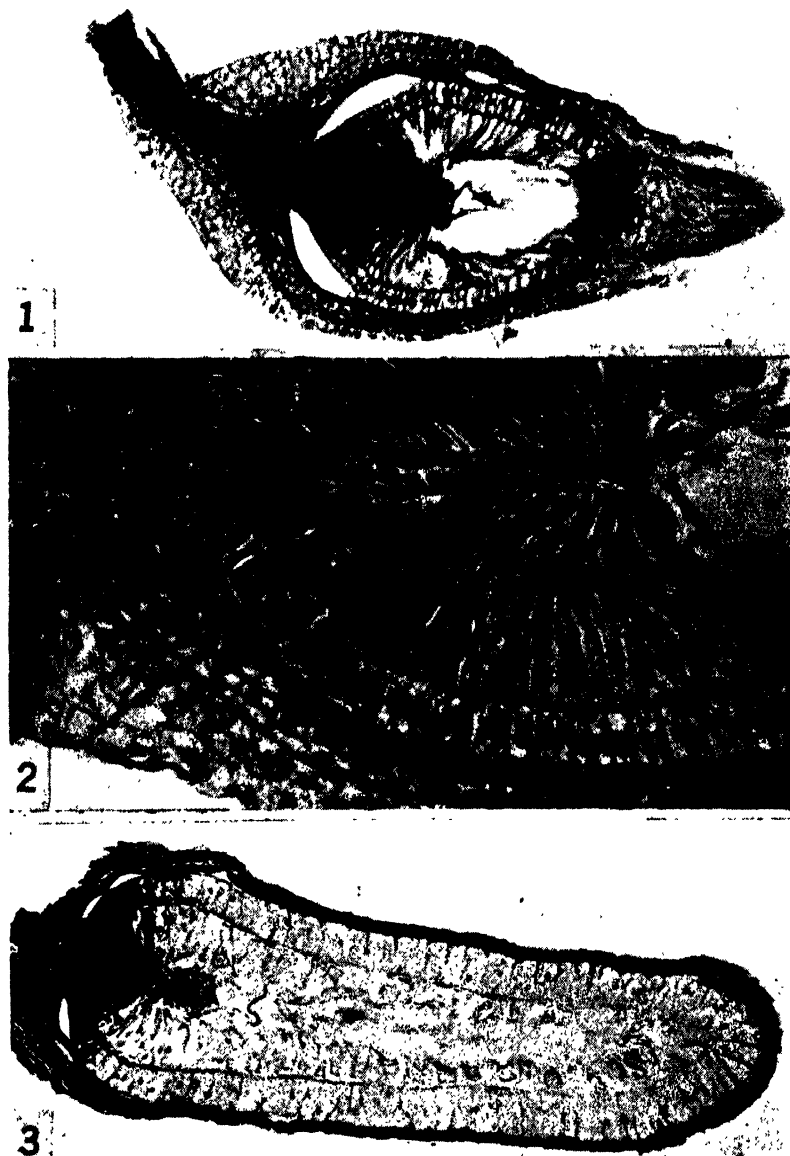
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is to be seen in the ovule at this phase, except that an axillary row of cells just back of the archesporial cell indicates the beginning of a conductive strand.

The inner integument grows rapidly, is two layers of cells thick, and when it has about closed over the end of the nucellus the ovule is but 0.2 mm. in length. It is followed directly by the growth of the somewhat more massive outer integument. As this outer integument begins to grow up around the inner, the latter forms a pluglike growth of intertwining cells. This plug for a time projects beyond the apex of the young ovule (fig. 1). Finally, in the ripe seed the outer integument grows out beyond it in finger-like outgrowths which inclose it like a pair of paws.

#### NUCELLUS

The epidermal layer of the nucellus rather early undergoes a periclinal division, forming a two-layered tissue about the megasporos. Until the two-nucleate stage of the megagametophyte, and even later, the cells of the outer layer remain more or less cubical in shape. The cells of the inner layer become elongated in a radial direction, however, all tending to point toward the chalazal megaspore. These cells extend somewhat back of the megaspore and surround the conducting strand which traverses the chalaza, where they undergo the most extreme development. When the megagametophyte is but two-nucleate, these cells are decidedly elongated and wedge-shaped, their narrow ends being toward the center. The protoplasm with the nucleus is concentrated toward the outer, larger end of the cell, while the narrower inner end is occupied by a more or less homogeneous substance that does not readily take the haematoxylin stain. The outer portions of these cells increase considerably in size as the ovule matures. When the megagametophyte is in the two-nucleate phase, they are 14  $\mu$  in length; by the time the polar nuclei fuse they are 36  $\mu$  in length; and later they may enlarge to more than three times the size they were at the two-nucleate stage. While the outer portion of these cells becomes enlarged and remains densely protoplasmic, the inner portion is drawn out, becomes strongly tapering, and the radiate appearance is considerably accentuated (fig. 2). The walls at the narrow inner ends of the cells



FIGS. 1-3.—Fig. 1, section of ovule showing conducting strand in funiculus and outgrowth of integuments at the micropyle. Fig. 2, chalazal end of ovule showing axillary strand passing through inner nucellar tissue to antipodal end of megagametophyte. Conspicuous bricklike layer of outer nucellus matures into the perisperm. Fig. 3, ovule at time of fertilization. Note wall across megagametophyte dividing it into small basal and a large micropylar cell. Fig. 1,  $\times 140$ ; fig. 2,  $\times 340$ ; fig. 3,  $\times 100$ .

become thickened and eventually no protoplasmic contents can be seen in this area.

During later development, the outer protoplasmic portion of these cells is absorbed by the enlargement of the megagametophyte. The thickened walls of their inner parts remain about the conducting strand and form the so-called postament, which projects into the older megagametophyte (fig. 3) and remains even in the mature seed, where it is imbedded in endosperm. The other cells of the inner layer of the nucellus (those toward the micropyle) increase in size and appear somewhat disarranged. In the mature seed this inner nucellar layer has been crushed except for that portion lying around the sides of the postament.

The vascular bundle of the funiculus reaches the chalazal part of the ovule, in which it ends rather abruptly (fig. 1). Beyond this point there is a region in the uppermost part of the chalaza and the very base of the nucellus in which the cells are undifferentiated and parenchymatous. This is continued upward as a narrow strand of parenchymatous cells which forms the axis of the postament, where it is two cells in thickness and probably persists by virtue of its protection within the hardened postament (fig. 2).

The epidermal layer of the nucellus develops differently from that of the inner layer just discussed. These cells gradually become prismatic and finally in the mature seed form the more or less truncated wedge-shaped cells of the perisperm. When the megagametophyte is two-nucleate these cells are for the most part cubical; at this time they are about  $3.5\ \mu$  on a side. While the megagametophyte is developing to the eight-nucleate stage they enlarge considerably, particularly in the radial direction. This growth is most marked toward the apex of the ovule, where the cells become about  $16\ \mu$  long by  $7\ \mu$  wide. This outer nucellar layer becomes very conspicuous and appears like a wall of bricks surrounding the inner structure of the ovule. At the chalazal end this layer is interrupted by the small region of more or less undifferentiated chalazal tissue (fig. 2). During the rapid growth of the ovule following fusion of the polar nuclei, anticlinal division occurs, since the number of epidermal cells visible in a single plane doubles during this period.

When the pollen tube enters, the cells of this layer are becoming

filled with a clear, transparent substance. This development is slowest at the micropylar end, the cells there retaining their normal protoplasmic appearance until after the pollen tube has passed through the tissue.

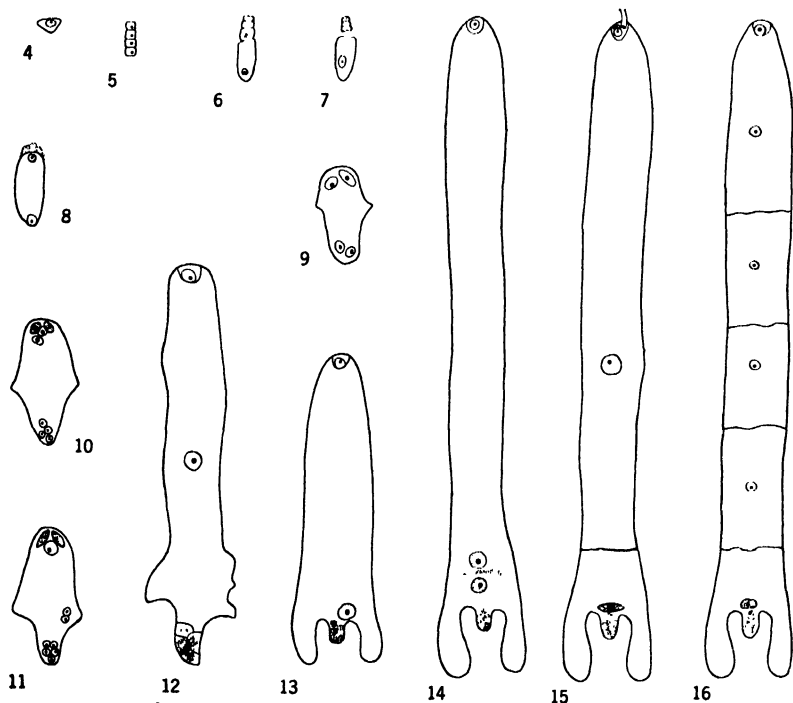
MÜCKE discussed this tissue in the mature seed of *Acorus gramineus* and concluded that the substance, which in the ripe, dry seeds is glassy, hard, homogeneous, and fills the cells completely, is cell contents and not cell wall material. There is nothing in the mature seeds to offer morphological proof of this. His conclusions were based on the chemical nature of the substance, which he proved to be protein. Sections showing the development of the perisperm confirm MÜCKE's interpretation. Soon after this substance appears in the cells, their radial walls become wavy, as are the thin membranes mentioned by MÜCKE in the mature perisperm of the seed of *A. gramineus*. Plasmolysis, which occurs very readily in the younger stages of development of the cells, shows plainly that the material in question is cell contents and not cell wall. Further, the nucleus may often be seen imbedded in cytoplasm of a cell well permeated with this substance. As the perisperm matures the nucleus disappears entirely, and the contents of the cells become uniform throughout.

From the time of fertilization, when the perisperm cells are about  $57 \times 32 \mu$  in radial section, to the mature seed, they enlarge enormously, attaining a measurement of about  $230 \times 60 \mu$  in radial section. They are roughly in the form of hexagonal prisms curved so that their inner ends all tend to point toward the chalaza.

#### DEVELOPMENT OF MEGAGAMETOPHYTE

A semidiagrammatic representation of the megagametophyte development is shown in figures 4-16. The archesporial cell which appears in the apex of the nucellus gives rise to an axial row of four megasporocytes, the chalazal one of which develops into the megagametophyte. The two-nucleate megagametophyte is about  $40 \mu$  long, and shows strong polarity, a large vacuole occupying the center. When the megagametophyte has enlarged to about  $70 \mu$  in length, it contains four nuclei. It begins its enlargement at the expense of the inner nucellar layer in the region near the micropyle, where a

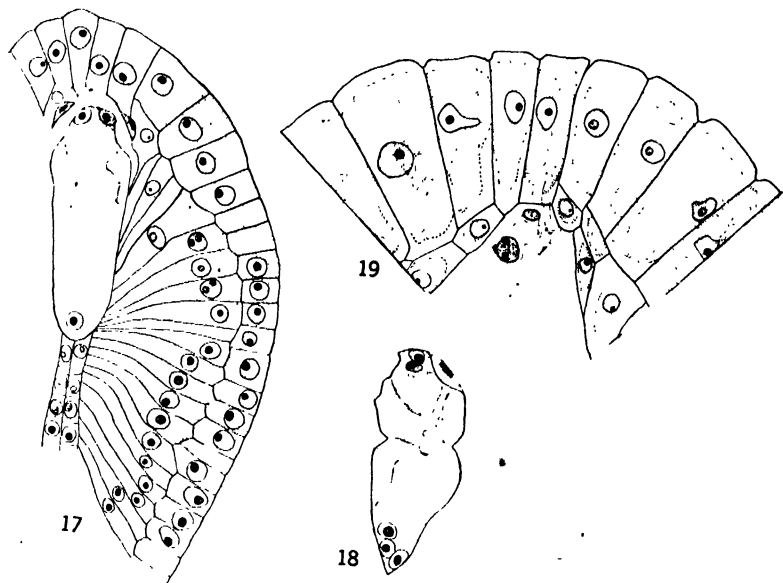
pocket-like cavity develops. Later the egg apparatus lies in this cavity. Without the megagametophyte having undergone any conspicuous enlargement, the four nuclei divide simultaneously.



FIGS. 4-16.—Fig. 4, archesporial cell. Fig. 5, tetrad of megaspores. Figs. 6, 7, degeneration of micropylar megaspores and enlargement of functional chalazal megaspore. Figs. 8-11, development of megagametophyte up to time of fusion of polar nuclei. Fig. 12, rapid elongation and enlargement of megagametophyte; synergids have disappeared. Fig. 13, postament formed and fusion nucleus moved to a point just over antipodals. Fig. 14, fusion nucleus dividing near base of megagametophyte. Fig. 15, fertilization taking place after a nucleus has been cut off in a basal cell. Fig. 16, commencement of endosperm formation. Figs. 4-12,  $\times 250$ ; figs. 13-15,  $\times 100$ ; fig. 16,  $\times 70$ .

In eight-nucleate megagametophytes the two synergids are for a time very conspicuous by virtue of their dense nucleoplasm and the dense cytoplasm directly surrounding the nuclei (fig. 17). Adjacent to these are darkly staining disorganized cells of the inner nucellar layer, at whose expense the megagametophyte has increased in size.

A physiological function connected with the disorganization of adjacent cells has been ascribed to such synergids (4). That the synergids in this case perform such a function seems probable, since as soon as the few cells that are resorbed at the apex of the megagametophyte are entirely gone, the synergids themselves begin to disorganize. This degeneration of synergids is well under way when the polar nuclei fuse, and soon after this the synergids disappear en-



FIGS. 17-19.—Fig. 17, eight-nucleate megagametophyte and two layers of nucellus cells. Fig. 18, section of same eight-nucleate megagametophyte as in fig. 17. Fig. 19, apical portion of megagametophyte and adjacent nucellar layers at time of fertilization. Figs. 17, 18,  $\times 389$ ; fig. 19,  $\times 439$ .

tirely. The egg nucleus is conspicuous and lies at the extreme micropylar end of the megagametophyte. Adjacent to it may be found for a time a polar nucleus (fig. 18). At the antipodal end are the three nuclei destined to remain as antipodals, together with the one polar contribution from that end. The polar nuclei migrate to the center or to a point near the center of the megagametophyte. This migration of the polar nuclei and their subsequent fusion is a rapid process. The megagametophyte with eight nuclei measures about  $65\ \mu$  in length and shows no signs of marked increase in width.

The beginning of rapid enlargement of the megagametophyte is concurrent with the fusion of the polar nuclei. It commences laterally and toward the base at the expense of nucellar and chalazal tissue. The nucellar cells begin to collapse around the middle portion of the megagametophyte. They look very much as if they were being crushed mechanically, the pressure being localized near the center of the ovule. This may possibly be explained by the differential hardening of tissues at the time this growth begins, since this point or region where enlargement is taking place is just above a modified "hardened" area. This area consists of the attenuated inner ends of the cuneate cells of the inner nucellar layer about the antipodal end of the megagametophyte. Basal enlargement of the megagametophyte continues by the resorption of the softer parts of the nucellus around this more resistant tissue which immediately surrounds the conducting strand. This results in the peculiar formation of the postament.

After the polar nuclei have fused, a marked elongation of the micropylar half of the megagametophyte is accompanied by rapid growth of the surrounding tissues, which is due in part to increase in number of cells, but also to the rapid increase in size of the individual cells themselves. The megagametophyte triples its length before the postament itself is evident at all. In an ovule which is ready for fertilization the megagametophyte measures about  $675\ \mu$  in length. Shortly after fertilization, when the endosperm has begun to form, the ovule is nearly double this size, and the embryo sac is  $1340\ \mu$  long. It continues to increase, until in the mature seed the endosperm with its inclosed embryo measures about 2 mm. in length.

When the polar nuclei have fused, the conspicuous fusion nucleus remains at first near the center of the cell. Through differential growth of the megagametophyte this nucleus appears farther and farther from the center and proportionately nearer the antipodal end of the megagametophyte. Finally, before dividing, it actually does move downward so that it comes to lie adjacent to the antipodals.

By division of one or two of the antipodals the number is increased, so that in some instances there are five. In these appear a number of large, irregular, deeply staining granules. The whole



nucleus eventually stains very dark with haematoxylin, while the conspicuous dense granules are still present. Thin cell walls form about these nuclei but no further development occurs. As the megagametophyte matures they occupy a small cavity at the apex of the postament, which projects into the megagametophyte, this cavity being the constricted antipodal end of the younger megagametophyte.

After the fusion nucleus moves to the basal end of the megagametophyte, division takes place immediately. This mitosis is followed by the formation of a cell plate which divides the megagametophyte transversely into two parts, a small basal cell and a larger one comprising the main body of the megagametophyte (fig. 3). As to the general appearance of the megagametophyte at this time, both resulting cells have a relatively small amount of protoplasm in comparison with the size of the vacuole. Around the postament the cytoplasm is denser than elsewhere, and in this, just over the antipodals, lies the lenticular nucleus of the basal cell. The other daughter nucleus, that which occupies the larger, main part of the megagametophyte, moves toward the center of this cell.

#### FERTILIZATION

In one ovule sectioned, a flattened nucleus, obviously a sperm nucleus, appears closely appressed to a spherical nucleus which is undoubtedly the egg (fig. 19). The collapsed remains of a pollen tube which has passed through the nucellus just above the egg apparatus are present in the same section. Near the end of this pollen tube there is a small body that may represent either the tube nucleus or a second sperm. It is noteworthy that the main body of the megagametophyte is already divided into two large chambers by a cross wall. There is no indication that a second sperm is to fuse with the large nucleus which occupies the center of the larger, micropylar chamber. In none of the ovules examined is there an instance where a sperm seemed to be fusing with this nucleus, and there is no indication of a sperm moving to it in the megagametophyte under consideration. Possibly the second sperm never reaches the megagametophyte.

The possibility that a second sperm nucleus is discharged from

the pollen tube raises an interesting question: does it affect the whole endosperm or only the micropylar tissue? Obviously if the megagametophyte cavity has been previously separated into two chambers following division of the fusion nucleus, the sperm nucleus can fuse only with the daughter nucleus in the micropylar chamber. Thus endosperm in the micropylar portion might be triploid while that arising in the basal chamber would be diploid. If there is such a functioning second sperm one answer to the question lies in knowing when it enters. Here the evidence, while falling somewhat short of complete demonstration, is convincing. In ovules with megagametophytes about  $300\ \mu$  long, taken from spikes in which pollination was actively occurring, there was no evidence of pollen tubes anywhere within the ovules. At this stage the fusion nucleus is lying at the base of the megagametophyte, adjacent to the antipodals.

On the other hand, by the time the megagametophyte has reached a length of  $700\ \mu$ , pollen tubes are conspicuously present, some of them still unopened within the nucellus, and others that appear to have recently discharged their contents into the megagametophyte. The ovule already described as showing fertilization is in this stage. In all such ovules the fusion nucleus has already divided, a wall has been formed across the lower part of the megagametophyte, and the upper endosperm nucleus has moved far up toward the middle. While intermediate stages have not been seen, it seems wholly unlikely that in the apparently brief interval between the entry of the pollen tube and the stage just described there would be time for the second male nucleus to traverse the whole length of the very long megagametophyte, for it to unite with the fusion nucleus, and then for the latter to divide and attain the stage just described.

The cytological condition of the endosperm should throw light on these problems, but it has proved very difficult to obtain preparations of endosperm showing mitotic figures. Although ovules were killed at various hours of the day and night in an attempt to secure division stages, only one mitotic figure has been seen, and that was in telophase with the spindle oriented parallel to the plane of the section, so that it was impossible to count the chromosomes.<sup>1</sup>

<sup>1</sup> The diploid number, determined by A. ORVILLE DAHL from sections of root tips, is 18.

## ENDOSPERM

It is fairly certain, then, that the nucleus in the basal cell of the embryo sac is diploid. That the nucleus in the main cell of the embryo sac is diploid rests only on negative evidence. The tissues resulting from these two nuclei, however, are somewhat different.

In the basal cell the nucleus is rather dense, which is likewise true of the cytoplasm immediately surrounding it. This nucleus does not undergo further development immediately. The one in the larger, micropylar cell, however, by a series of divisions followed by transverse walls produces a row of cells down the center of the embryo sac. It cannot be said, in the light of the present study, whether the endosperm formation in this upper cell is initiated by free nuclear division or whether it is cellular from the start, but it is obvious that if a stage of free nuclear division occurs it is very brief. Transverse divisions continue for some time in the main part of the endosperm, so that several transverse chambers are produced. Soon, however, these are broken up into irregular cells by the formation of oblique and longitudinal walls. The endosperm ultimately is composed of uniform small cells with moderately substantial walls. It is formed rapidly and comes to occupy practically all the space inside the perisperm, enlarging at the expense of the inner nucellar cells. It is completely developed while the embryo is still young. The center of it is then digested away by the growing embryo.

Meanwhile the basal cell formed by the first division of the fusion nucleus remains unchanged for some time, at least until four cells have been formed in the upper part of the embryo sac. At length it divides repeatedly and gives rise to a more or less dense mass of irregular cells that push in around the postament and cover its upper end. For a time this tissue is conspicuously different from the rest of the endosperm, having smaller and less vacuolate cells and denser nuclei; it gradually loses its distinctive appearance as the seed matures. The original septum that cuts off this part of the embryo sac becomes difficult to distinguish in later stages of seed development, but traces of it can be noted even in the mature seed (2).

## EMBRYO

The embryo develops somewhat more slowly than the endosperm. The two-celled embryo lies in an embryo sac in which the endosperm consists of a series of transverse cells. The first division of the zygote is transverse to the axis of the embryo sac. The upper cell then divides in a plane parallel to this. Usually each of the resulting three cells undergoes a transverse division, following which longitudinal divisions first occur. Development up to this point is not rigid, however, as the initiation of longitudinal walls may occur sooner with the formation of a shorter, stubbier proembryo. The basal cell is larger than the others and does not appear to divide more than once after it is first formed by the division of the zygote.

The embryo now rapidly becomes pyriform, all the cells but the basal one undergoing one or more longitudinal divisions, the apical cells producing most of the growth. By the time the embryo is 0.55 mm. long, differentiation is already apparent. The plumule is evident and the procambial strand can be seen in the cotyledon and extending down into the hypocotyl. The embryo continues to enlarge, utilizing the already formed endosperm as it grows up through its center. The mature embryo finally occupies the axis of the elongate endosperm and extends for about three quarters or more of its length. In the mature seed it is about 1 mm. long. At its base is a short cylindrical suspensor. Although this is frequently not obvious in longitudinal sections, it occasionally shows up clearly at the base of the mature embryo.

## Summary

1. Except for the early division of the fusion nucleus followed by the formation of a cross wall dividing the megagametophyte into two large chambers before fertilization, the development of *Acorus calamus* follows the fundamental type (Normaltypus of SCHNARF 7).
2. The endosperm is of the basal apparatus type. In this type the daughter nucleus, which remains at the base of the megagametophyte after the first division of the fusion nucleus, produces a tissue different in character from the other nucleus which gives rise to the main part of the endosperm. With very few exceptions the Araceae are characterized by this type of endosperm.

3. The particularly interesting feature about *Acorus calamus* is the division of the fusion nucleus before the pollen tube enters and the consequent segregation of a diploid nucleus in the basal cell before fertilization occurs.

I wish to thank Dr. F. K. BUTTERS and Dr. C. O. ROSENDAHL, who suggested the problem and gave their valuable advice during the course of this work; and Dr. R. R. HUMPHREY and Dr. E. L. NIELSEN for their aid in the collection of material. This research was done in the botanical laboratories of the University of Minnesota.

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# STIMULATING EFFECT OF BETA(3)INDOLEACETIC ACID ON SYNTHESIS OF SOLID MATTER BY BEAN PLANTS<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 489

JOHN W. MITCHELL AND CHARLES L. HAMNER

(WITH FIVE FIGURES)

## Introduction

Recent investigations have shown that hormones can alter the growth rate of roots and shoots (1, 7, 8, 9, 10), affect the morphological and histological development (2, 3), and to some extent control the transfer of some carbohydrate and nitrogenous compounds (5) within certain plants grown in darkness. In connection with these investigations, it was considered desirable to study the effect of beta(3)indoleacetic acid on the assimilation of solid matter by plants. For this purpose several thousand bean plants were grown under greenhouse conditions, and the fresh and dry weights of different portions of treated and untreated plants were compared at the beginning and end of given periods following treatment.

## Investigation

**MATERIALS AND TREATMENT.**—Kidney beans, *Phaseolus vulgaris*, were used as experimental material. In each experiment several thousand seeds were selected for size and uniformity and planted at an even depth in quartz sand, or in the case of preliminary experiments in soil contained in 5 inch clay pots. They were watered with warm tap water and placed in a greenhouse at 75°–80° F. The relative humidity varied from approximately 40 to 80 per cent, but was generally above 60 per cent. The light intensity varied from approximately 200 foot candles, on very dark cloudy days, to approximately 5000 foot candles on clear days in early November, and 2000–3000 on clear days during December and January.

<sup>1</sup> This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation..

The plants were usually 3-4 inches above the sand level on the seventh day after planting. The sand was flushed with nutrient (6) and seed coats were removed so that further development was not impeded. After ten days the plants were 7-9 inches high and the second internode from 1 to 1.5 inches in length.

A number of uniform plants were then selected sufficient to allow for approximately 225 plants in each treatment, including initial and final controls. A tag, marked to correspond to the particular treatment, was placed in each pot. In this way any individual treatment was scattered through the entire population.

The 2 per cent indoleacetic acid lanolin mixtures used in some experiments were prepared by adding 100 mg. of indoleacetic acid (Merck) to 5 gm. of anhydrous lanolin contained in a small vial. The lanolin was melted and the mixture thoroughly stirred until it cooled and solidified.

Various other concentrations of indoleacetic acid were prepared by dissolving the acid in 95 per cent ethyl alcohol. The desired concentration was then prepared by adding the correct number of drops of the alcoholic solution to a weighed portion of lanolin, with the aid of a pipette. To prepare a more dilute mixture, the original alcoholic solution was diluted 1 volume to 9 with 95 per cent alcohol, and the same number of drops of the resulting solution added to another portion of lanolin of the same weight. This process was repeated until a series of concentrations was obtained. The alcohol, which remains on the surface of the lanolin, was immediately evaporated by means of a stream of warm air, and the indoleacetic acid was finally stirred into the mixture as previously mentioned.

Prior to treatment, the stems of approximately 225 plants were severed at the second internode 1.5 cm. above the point of divergence of the primary leaves. These plants were then harvested and designated as initial controls. The stems of another group were severed in like manner and 3-4 cu. mm. of pure lanolin was applied to the freshly cut surface. These were designated as final controls and harvested at the end of the experiment together with the treated plants. Stems of remaining plants were severed and 3-4 cu. mm. of the desired concentration of the lanolin mixture applied to the cut

surface. Following treatment, the pots were flushed with nutrient solution every third day and moistened with tap water on intervening days.

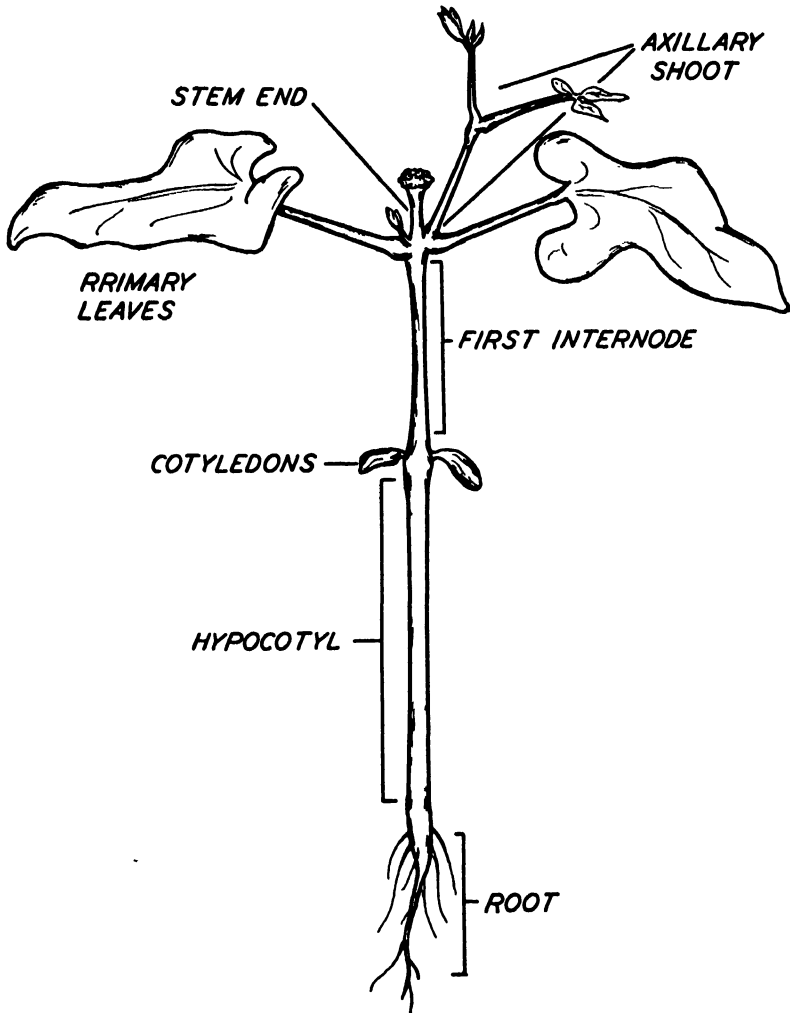


FIG. 1.—Parts into which plants were divided for weight determinations

In some experiments it was desirable to prevent the growth of new shoots which developed in the axils of the leaves soon after the stems were severed. The newly developed axillary buds, 1–3 mm.



in length, were therefore carefully removed and discarded at intervals during the experiments.

**DETERMINATION OF FRESH AND DRY WEIGHTS.**—At harvest the roots were first washed free from most of the sand, then dipped into a saturated solution of sodium chloride for approximately 30 seconds to loosen the remaining sand. The roots were immediately washed and the plants carefully divided into the following fractions: (1) roots, (2) hypocotyls, (3) cotyledons, (4) first internodes, (5) primary leaves and petioles, (6) axillary shoots together with leaves and petioles, and (7) the treated portion of the second internode later designated as stem end (fig. 1).

The fresh weight of each fraction representing the parts of approximately 225 plants was recorded. The fractions were chopped fine and placed in a well ventilated drying oven at 80° C. for a period of 24–30 hours. The dry weight of each fraction was then determined by means of a torsion balance which was sensitive to 0.05 gm., or in the case of small samples, by means of an analytical balance.

The leaf areas of some of the plants were measured by means of a photoelectric method (4).

In all experiments the results were obtained from samples representing approximately 225 plants. Previous experiments (5) with plants grown in the dark and also preliminary experiments with plants grown under greenhouse conditions showed that duplicate samples from this number of plants did not differ more than 3 per cent in dry weight. As a matter of convenience the data are presented on the basis of 100 plants.

### Results and discussion

**MORPHOLOGICAL OBSERVATIONS.**—The first apparent response of plants to concentrated mixtures (0.2–2.0 per cent) of indoleacetic acid occurred within 48 hours after treatment. The stems changed from dark to light green for a distance of 3–5 mm. below the point of application. Tumors and roots, similar to those observed in previous experiments (3), later developed directly below the treated surface. When more dilute concentrations were used (0.002 per cent or less), there was less change in the color of the stem ends and no

noticeable enlargement of the stem below the point of treatment. Transverse sections through this region of plants treated with 0.000185 and 0.0000185 per cent of indoleacetic acid showed that roots were not initiated during a period of six days. However, plants treated with the most dilute concentrations formed more callous tissue at the cut surface than did control plants treated in a similar manner with pure lanolin. The amount of proliferation of tissues at the stem end varied with the lighting conditions, as relatively small tumors were produced by treated plants during dark cloudy days of December and January.

TABLE 1

DRY WEIGHT GAINED BY PARTS OF UNTREATED PLANTS  
AS COMPARED WITH THOSE OF PLANTS TREATED  
WITH 2 PER CENT INDOLEACETIC ACID. FIGURES  
REPRESENT GRAMS SOLID MATTER GAINED PER 100  
PLANTS DURING PERIODS OF FIVE DAYS (EXPERI-  
MENT 1) AND SIX DAYS (EXPERIMENT 2)

PARTS	CONTROL	TREATED
Experiment 1		
Leaves.....	9.6	9.7
Axillary shoots.....	12.6	5.7
Stem ends.....	0.68	2.89
Experiment 2		
Leaves.....	14.7	15.8
Axillary shoots.....	17.6	8.5
Stem ends.....	1.06	4.15

Preliminary experiments showed that plants treated with concentrations greater than 0.002 per cent developed smaller axillary shoots than did control plants. No noticeable difference, however, was observed between the growth of axillary shoots of control plants as compared with those of other plants treated with concentrations less than 0.002 per cent. There was no apparent difference in the size and color of the primary leaves of treated and untreated plants.

FRESH AND DRY WEIGHTS.—As initial experiments, plants grown in soil were treated with 2 per cent indoleacetic acid lanolin mixture. Control plants, together with an equal number of treated plants, were harvested at the end of five or six days following treatment.

The dry weights of primary leaves, stem ends, and axillary shoots were determined. The results show that indoleacetic acid caused solid matter to move toward the point of application, and that development of the axillary shoots was definitely retarded owing to its application (table 1).

More extensive experiments were then conducted, first to study the effect of indoleacetic acid on the dry weight of different parts of the entire plant, during an extended period of treatment, and

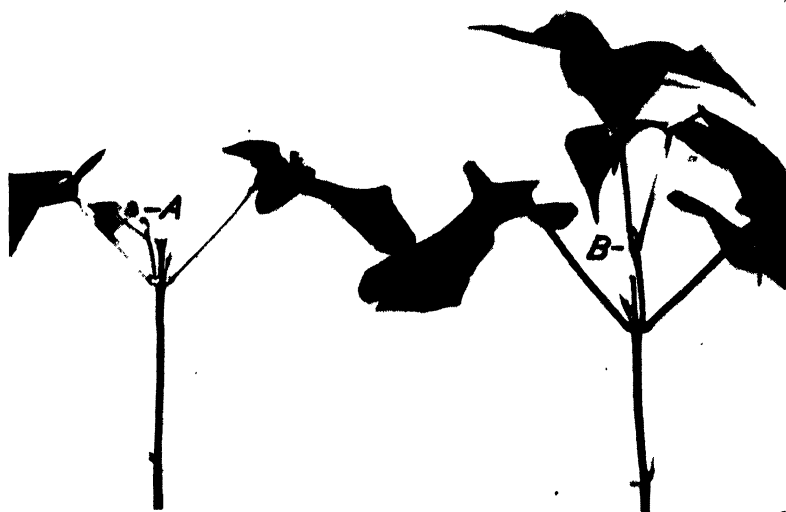


FIG. 2.—Growth of axillary shoots of untreated plant (B) compared with that of plant treated with 2 per cent mixture of indoleacetic acid and lanolin (A). Picture taken eight days after treatment.

second to determine its effect on the total amount of solid matter synthesized by plants during a given period.

For this purpose plants grown in sand were treated with 2 per cent indoleacetic acid lanolin mixture. Controls, together with an equal number of treated plants, were harvested at frequent intervals during the following period of fifteen days.

During the first three days following treatment one or two dormant buds in the axils of the primary leaves of control plants became active and grew rapidly, while on the other hand the stem end of treated plants showed active growth and the axillary buds re-

remained dormant. Thus during this period the axillary buds of control plants gained approximately 1.7 gm. of solid matter and the stem ends of similar plants gained only 0.2 gm. per 100 plants. In contrast to this, the axillary shoots of treated plants gained only 0.6 gm. and the stem ends of treated plants gained 1.1 gm. of solid matter per 100 plants (fig. 2, table 2).

From the third to the sixth day following treatment the axillary shoots of controls grew rapidly, withdrawing practically all neces-

TABLE 2

COMPARISON OF GAIN IN DRY WEIGHT OF PLANTS TREATED WITH 2 PER CENT INDOLEACETIC ACID WITH THAT OF UNTREATED PLANTS. FIGURES REPRESENT GRAMS DRY WEIGHT PER 100 PLANTS

PARTS	3 DAYS		6 DAYS		9 DAYS		15 DAYS	
	CONTROL	TREATED	CONTROL	TREATED	CONTROL	TREATED	CONTROL	TREATED
Stem ends.....	0.203	1.143	0.447	2.752	0.689	4.259	1.009	5.179
Axillary shoots..	1.691	0.670	10.7	3.76	29.8	13.1	60.6	26.9
Primary leaves..	9.90	7.48	5.82	9.82	12.75	13.72	5.94	7.17
First internodes.	0.88	1.02	1.39	2.25	1.88	2.80	1.94	2.91
Hypocotyls.....	1.69	2.10	1.24	2.14	1.39	2.95	3.98	2.76
Roots.....	4.34	3.96	3.55	3.91	5.43	5.23	7.20	6.65
Total.....	18.70	16.38	23.15	24.63	51.93	42.06	80.67	51.57
Total minus axillary shoots....	17.01	15.71	12.45	20.87	22.13	28.96	20.07	24.67

sary nutrients from the other parts of the plants, as the leaves of the young shoots were not yet expanded. This is shown by the fact that the axillary shoots of 100 control plants gained approximately 9 gm. of solid matter while the remainder of the same plants lost 4.5 gm. during this period. The leaves of the young shoots of control plants expanded and became active photosynthetically soon after the sixth day following treatment, and all parts of the plants showed a gain in solid matter following that date. In contrast to this, the growth of axillary shoots on treated plants was inhibited and solid matter moved largely toward the point of treatment instead of into the axillary shoots.

During the six days following treatment, both treated and control

plants were largely dependent upon their primary leaves (which were of approximately the same area) as a source of carbohydrates. The effect of indoleacetic acid on the total amount of solid matter synthesized by treated and untreated plants during this time can therefore be compared on the basis of approximately equal leaf areas. Such a comparison shows that during the first three days, indoleacetic acid slightly retarded the synthesis of solid matter, but from the third to the sixth day 100 treated plants gained approximately 8.3 gm. of solid matter while the same number of untreated plants gained only 4.5 gm. Thus indoleacetic acid not only causes an accumulation of solid matter near the point of application, but it also stimulates the synthesis of solid matter by the plant. This stimulation in rate of synthesis of solid matter was not evident after approximately the sixth day, however, as the control plants developed new axillary shoots and leaf surface more rapidly than did treated plants.

In general the results show: (1) that extension of the stem axis and development of new leaves was retarded by the treatment (fig. 2, table 2); (2) that those parts of treated plants nearest the point of application (stem ends, primary leaves, and first internodes) gained more solid matter than did similar parts of control plants; and (3) that the total gain in solid matter by treated plants was at first slightly less than that of controls, but between the third and sixth day the treated plants gained approximately twice as much solid matter as did the controls. After about the sixth day the control plants accumulated weight more rapidly, probably because of their greater leaf area than that of the treated plants. Variations in fresh weights of treated and untreated plants were similar to those observed in the case of the dry weights.

Further experiments were conducted to study in detail the effect of different concentrations of indoleacetic acid on the rate at which plants synthesized solid matter. The factor of bud inhibition was eliminated in order to simplify the experiment, and to determine definitely whether indoleacetic acid affected the amount of solid matter synthesized, irrespective of its effect on bud development.

Plants were treated with various concentrations of indoleacetic

acid, newly developed axillary buds removed from both treated and control plants, and weight determinations made at the end of the following six day period. The experiment was repeated four times during the months of December and January.

On the basis of 100 plants, those grown during the interval of December 4-10 and treated with 0.000185 per cent indoleacetic acid

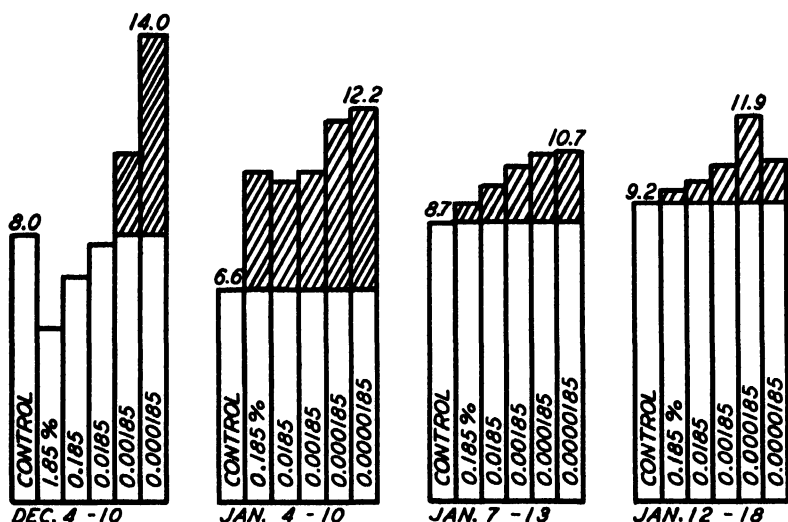


FIG. 3.—Gain in dry weight by four sets of plants, each group treated with indoleacetic acid lanolin mixtures covering similar range of concentrations. Plants of each group were of slightly different ages at time of treatment. Axillary shoots removed, leaving primary leaves as photosynthetic organs. Each column represents grams dry matter synthesized by 100 plants during six days. Shaded portions represent dry matter synthesized by treated plants in excess of that gained by controls.

gained 14.0 gm. of dry matter, while the same number of untreated plants gained only 8.0 gm. Similarly 100 plants grown between the fourth and tenth of January and treated with 0.0000185 per cent indoleacetic acid gained 12.2 gm. of solid matter, whereas a like number of untreated plants gained 6.6 gm. (fig. 3, table 3). The fresh weight of treated and untreated plants varied in approximately the same way. During these two experiments the average light intensity was relatively high. Thus the plants were stimulated in their ability to synthesize solid matter, when treated with a low concen-

tration of indoleacetic acid and grown during a period of relatively high light intensity. Treated plants of other groups grown during dark cloudy weather were much less affected (table 4).

Although the plants used for any single experiment were similar in size, appearance, and age, they varied between experiments at the

TABLE 3

RESULTS OF TREATING WITH DIFFERENT CONCENTRATIONS OF INDOLEACETIC ACID. FIGURES REPRESENT GRAMS DRY MATTER GAINED BY 100 PLANTS IN EACH OF FOUR EXPERIMENTS, DURING A PERIOD OF SIX DAYS

DATE OF EXPERIMENT	CONTROL	PERCENTAGE CONCENTRATION					
		1.85	0.185	0.0185	0.00185	0.000185	0.0000185
December 4-10...	8.03	5.32	6.99	7.99	10.74	14.02	.....
January 4-10....	6.62	.....	10.34	9.81	10.14	11.66	12.20
January 7-13....	8.67	.....	9.19	9.82	10.33	10.67	10.69
January 12-18...	9.19	.....	9.60	9.98	10.44	11.94	10.54

TABLE 4

EFFECT OF LIGHT INTENSITY ON RESPONSE OF PLANTS TO DILUTE CONCENTRATIONS OF INDOLEACETIC ACID. FIGURES REPRESENT MAXIMUM INCREASE IN GRAMS OF DRY WEIGHT PER 100 PLANTS OVER A PERIOD OF SIX DAYS

DATE OF EXPERIMENT	HOURS OF SUNSHINE	GAIN OVER THAT OF CONTROLS
December 4-10.....	32.5	6.0
January 4-10.....	24.6	5.6
January 7-13.....	17.6	2.0
January 12-18.....	17.1	2.8

time of treatment because of differences in light conditions. Thus plants of some experiments were relatively sturdy while others grown during periods of dark cloudy weather were etiolated.

Both the treated and the untreated plants were dependent mainly on the primary leaves for synthesis of carbohydrates, as new buds were removed when less than 3 mm. in length. Area measurements were made to study the effect of indoleacetic acid on the rate of

expansion of these primary leaves. Table 5 shows that 0.000185 and 0.0000185 per cent mixtures caused a slight stimulation in leaf expansion by plants grown during a period of relatively high light intensity. On the other hand, the leaf expansion of treated plants was inhibited by indoleacetic acid during periods of dark cloudy weather. It is evident that there is a relationship between light intensity and effect of indoleacetic acid on leaf expansion, but more detailed experiments are necessary to establish the nature of this relationship.

TABLE 5

EFFECT OF DIFFERENT CONCENTRATIONS OF INDOLEACETIC ACID ON EXPANSION OF LEAVES. FIGURES REPRESENT SQUARE CENTIMETERS OF LEAF SURFACE PER 100 PLANTS. MEASUREMENTS MADE ON SIXTH DAY FOLLOWING TREATMENT

DATE OF EXPERIMENT	CONTROL	PERCENTAGE CONCENTRATION				
		0.185	0.0185	0.00185	0.000185	0.0000185
January 4-10. . . . .	11460	11030	10950	11570	12120	12140
January 7-13. . . . .	13060	11360	11620	11580	11800	11800
January 12-18. . . . .	13750	12870	12550	12850	12900	12100

It is of importance to note that indoleacetic acid stimulated the plants to synthesize more solid matter, without causing a corresponding increase in leaf area.

It is evident from figure 4 and the data presented in table 6 that the amount of proliferation of stem tissue and the accumulation of solid matter near the point of application were proportional to the concentration of indoleacetic acid used. Thus the stem ends of 100 plants treated with 0.185 per cent mixture gained 1.36 gm. of solid matter during the interval from January 12 to 18, while those of the same number of plants treated with 0.0000185 per cent gained only 0.48 gm. during the same time. Although plants treated with concentrated mixtures showed a marked response near the point of treatment, this localized response was accompanied by a relatively small gain in the dry weight throughout the rest of the plant (fig. 5). Plants treated with more dilute concentrations showed practically



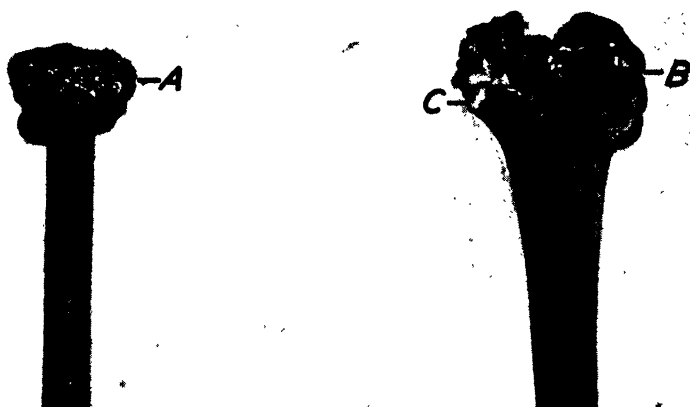


FIG. 4.—Tumor formation and root development by plant treated with 2 per cent mixture of indoleacetic acid and lanolin (B, C) as compared with callus formed by plant treated with a 0.0000185 per cent mixture (A). Picture taken six days after treatment.

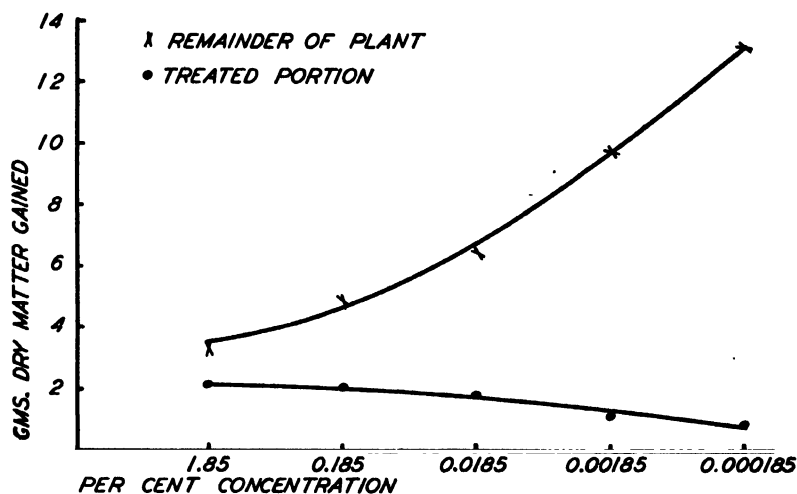


FIG. 5.—Decrease in concentration of indoleacetic acid lanolin mixtures from approximately 2 to 0.0002 per cent caused decrease in dry weight of tumors produced and marked increase in dry weight of remaining parts of plants. Figures represent grams dry matter gained per 100 plants during six days.

no response near the point of treatment, but gained more in fresh and dry weight than did plants grown under the same conditions but treated with concentrated mixtures of the acid.

In general it is concluded that indoleacetic acid is not only capable of inhibiting development of dormant axillary buds and causing the tissues of some plants to proliferate and form roots at the point of

TABLE 6

INCREASE IN DRY WEIGHT OF STEM ENDS OF PLANTS TREATED WITH VARIOUS CONCENTRATIONS OF INDOLEACETIC ACID. FIGURES REPRESENT GRAMS GAINED PER 100 PLANTS DURING A PERIOD OF SIX DAYS

EXPERIMENT	CONTROL	PERCENTAGE CONCENTRATION					
		1.85	0.185	0.0185	0.00185	0.000185	0.0000185
Dry weight							
December 4-10. . . . .	0.80	2.29	2.26	1.76	1.14	0.99	.....
January 4-10. . . . .	0.39	.....	1.28	1.19	0.87	0.54	0.46
January 7-13. . . . .	0.35	.....	1.57	1.49	1.08	0.47	0.56
January 12-18. . . . .	0.48	.....	1.36	1.24	0.86	0.55	0.48
Fresh weight							
December 4-10. . . . .	7.7	17.9	18.1	15.2	11.9	12.7	.....
January 4-10. . . . .	3.0	.....	9.0	8.2	6.0	4.5	3.6
January 7-13. . . . .	3.0	.....	12.5	12.4	8.8	5.0	5.1
January 12-18. . . . .	3.8	.....	8.9	8.5	6.2	5.2	4.4

application, but, particularly when used in small amounts, it is capable of stimulating the plants to synthesize a greater amount of solid matter.

### Summary

1. The stem tips of bean plants were removed and lanolin containing different concentrations of indoleacetic acid applied to the cut surface. When concentrations stronger than approximately 0.00185 per cent were used, the development of axillary buds was retarded, the stem ends produced tumors and roots, and those parts of the plants near the point of treatment increased more in fresh and dry weight than did similar parts of untreated plants.

2. When concentrations of indoleacetic acid lower than approximately 0.00185 per cent were used, its inhibitive effect on develop-

ment of axillary buds was less, the plants produced much smaller tumors, and no roots were developed.

3. By removing the axillary buds from both treated and untreated plants it was possible to study the effects of indoleacetic acid other than its inhibitive action on bud development. Dilute concentrations caused plants with the axillary buds removed to gain 23-85 per cent more solid matter as compared with untreated plants during a period of six days. The final dry weights of plants treated with these concentrations were between 6 and 11 per cent greater than the final weights of untreated plants at the end of a six day period, although the average weight of all of the plants was the same at the time of treatment.

4. Plants with axillary buds removed were stimulated to synthesize a much greater amount of solid matter when grown in light of a relatively high intensity than when treated with the same concentration of indoleacetic acid and grown during a period of relatively low light intensity.

5. The area of leaf surface of treated plants with axillary buds removed was not appreciably greater than that of untreated plants at the end of a six day period of treatment.

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# CYTOLOGICAL INVESTIGATIONS OF PISUM SATIVUM

GEORGE OLDS COOPER<sup>1</sup>

(WITH TWENTY-EIGHT FIGURES)

## Introduction

Little, if any, detailed study has been made of either microsporogenesis or megasporogenesis in *Pisum sativum* Linn. HÄKANSSON (6, 7), PELLEW and SANSOME (9), RICHARDSON (10), and SANSOME (12, 13) were particularly interested in the peculiar types of chromosome associations found at diakinesis and on the heterotypic equatorial plates of certain partially sterile plants. CANNON (1) described the behavior of chromosomes in pea hybrids. Investigations in the *Vicia* tribe of the Leguminosae have been concerned chiefly with the embryo development of various species of *Lathyrus*. LATTER (8) made a study of microsporogenesis for *L. odoratus*. FISK (5) determined the haploid chromosome number to be seven for a number of species of the same genus. ROY (11) reported the development of the megagametophytes of several members of the Leguminosae, including *P. sativum*. His investigation of this species indicates that the archesporial cell functions directly as the megaspore mother cell and does not cut off the parietal cell.

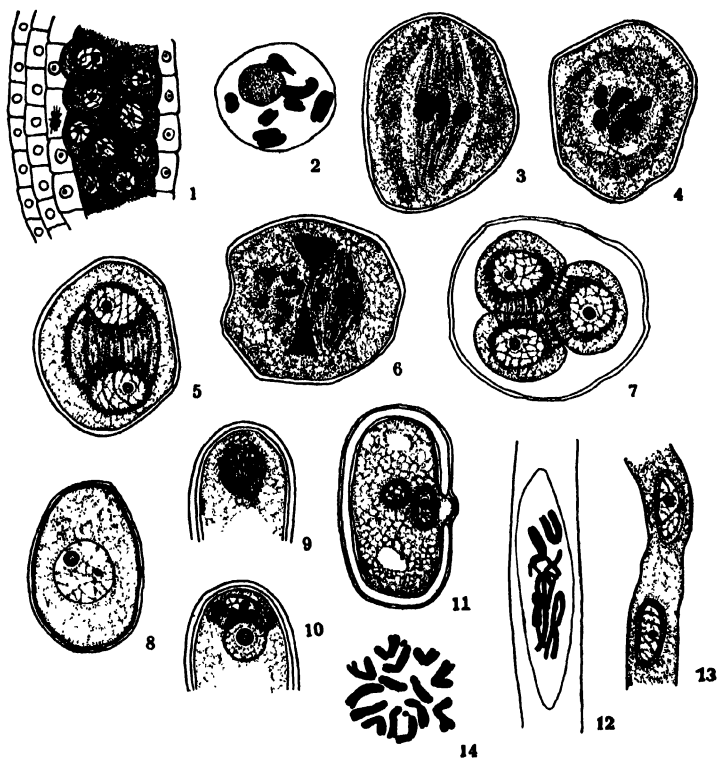
**MATERIAL AND METHODS.**—The material used in this investigation was from two horticultural strains of the garden pea (Little Marvel and Asgrow's Pride). Buds of various ages were fixed either in Karpechenko's modification of Navashin's solution or in Carnoy's solution, imbedded in paraffin, sectioned at 12  $\mu$ , and stained in Delafield's haematoxylin.

## Investigation

**MICROSPOROGENESIS.**—Each theca of the anther contains many microspore mother cells, which are readily identified because of their

<sup>1</sup> The writer wishes to express his appreciation to the Departments of Genetics and Botany, University of Wisconsin, for the facilities made available during the course of this investigation.

size and staining qualities (fig. 1). The nuclei of the tapetal cells which surround the sporogenous tissue divide during the interval



FIGS. 1-14.—Microsporogenesis (all  $\times 1000$  unless otherwise indicated): Fig. 1, longitudinal section through anther showing sporogenous tissue;  $\times 550$ . Fig. 2, diakinesis, seven pairs of chromosomes;  $\times 1250$ . Fig. 3, first meiotic metaphase. Fig. 4, polar view. Fig. 5, interkinesis. Fig. 6, second meiotic metaphase; spindles at right angles. Fig. 7, cytokinesis; partition walls appearing. Fig. 8, mature microspore. Figs. 9, 10, divisions of microspore nucleus. Fig. 11, young pollen grain. Fig. 12, late prophase and division of generative nucleus;  $\times 1450$ . Fig. 13, two male gametes in pollen tube;  $\times 1450$ . Fig. 14, polar view, somatic division, fourteen chromosomes;  $\times 1250$ .

in which those of the sporogenous cells are undergoing the prophase stages of meiosis. Each tapetal cell ultimately becomes binucleate. LATTER records the same behavior for *Lathyrus odoratus*. A marked

enlargement of the microspore mother cell nucleus occurs during the late prophase stage. Seven pairs of chromosomes are present at diakinesis (fig. 2). The chromosomes take a dense stain at this stage whereas the nucleole stains faintly. Polar views of somatic equatorial plates in root tip cells show fourteen chromosomes. Two of the chromosomes possess prominent satellites. The chromosomes are slender and variable in length, bent at the points of spindle attachment constrictions, and show arms of unequal length (fig. 14).

Immediately following diakinesis the nucleus shrinks somewhat in size, the nucleole disappears, the nuclear membrane disintegrates, and the chromosomes are massed near the center of the nuclear cavity. A multipolar spindle, which ultimately becomes bipolar diarch, forms with the chromosomes arranged at the equator midway between the poles. The paired nature of the chromosomes is readily distinguishable at metaphase (fig. 3). The seven bivalent chromosomes are easily counted in polar views of the first meiotic metaphase (fig. 4). The cytoplasmic sheath observed by COOPER (2) and COOPER (3, 4) was present. The newly formed nuclei at interkinesis do not fully take on the characteristics of a resting nucleus, but remain temporarily in a late prophase condition (fig. 5). The spindles of the second meiotic metaphase are usually at right angles to each other, a dense zone of cytoplasm from the first meiotic division separating the two spindles (fig. 6). LATTER did not describe the presence of this dense perinuclear zone in *Lathyrus*.

Subsequently a cell containing four nuclei is formed as a result of the second meiotic division (fig. 7). The spindles of this division persist for a time and cell plates are formed midway between the nuclei of the tetrad. The cell plate splits and ultimately four spores are formed, each containing one nucleus.

The spores are released from the microspore mother cell wall shortly after their formation, and are free in the theca. The microspore becomes ovoid, increases in size, and a smooth exine is developed (fig. 8). The nucleus of the mature microspore takes a position at the end of the cell and divides mitotically. The spindle lies lengthwise in the cell (fig. 9). A cell plate is laid down across the spindle and the microspore is divided to form a large tube cell and a smaller generative cell (fig. 10). The tube nucleus disintegrates

early and at the time of pollination has practically disappeared. During the course of development of the pollen grain the intine has increased in thickness, forming a heavy wall except over the germ pores. Each pollen grain has two laterally placed germ pores. A large vacuole is found within each pore, covered with a thin intine. As a rule the generative nucleus does not divide until after growth of the pollen tube. The newly formed generative cell when young is slightly ovoid in shape, but on maturation of the microgametophyte becomes linear to sickle-shaped (fig. 11).

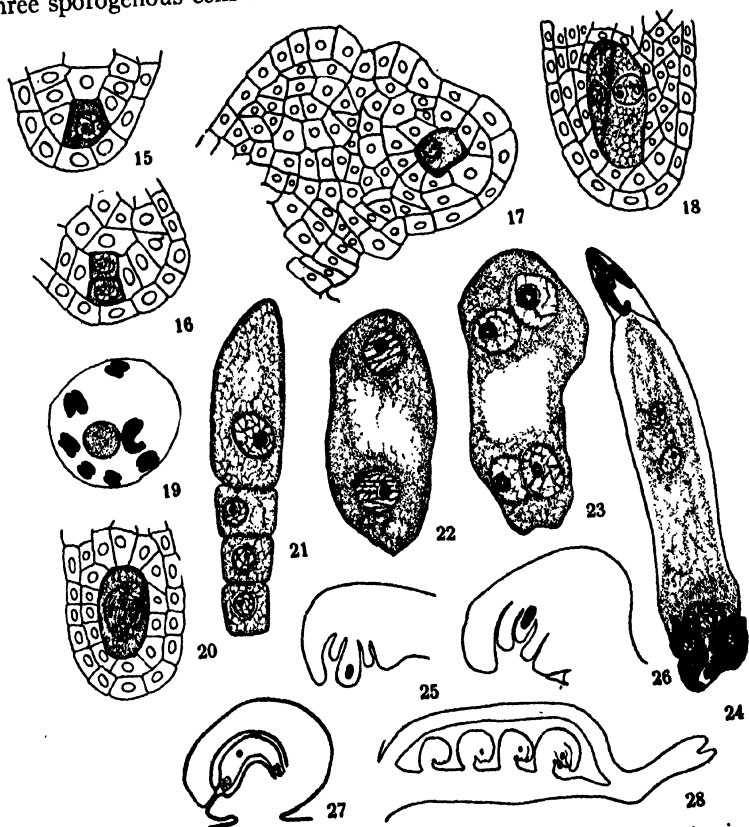
The pollen grains are shed before the opening of the flower. The chances of cross pollination are very slight, therefore, because when the flower opens the pollen tubes have progressed far enough down the style so that foreign pollen would be ineffectual. The generative cell divides mitotically while the pollen tube is growing down the style (fig. 12). Following this division the two male gametes are surrounded by a narrow ring of cytoplasm, a thin membrane separating them from the tube cytoplasm. The gametes are carried in the cytoplasm of the pollen tube and progress with it to the micropyle of the ovule (fig. 13).

OVULE DEVELOPMENT.—The flower contains a single ovary bearing typically ten ovules borne alternately on the two placentae. All of these may not be fertilized, or if so may not continue development, so that only rarely does one find ten ripened ovules in the mature fruit. When the megaspore mother cell is at the early prophase of the first meiotic division, two rounded outgrowths appear from the placental tissue. These outgrowths develop more rapidly on one side of the ovule than on the other and the ovule bends toward the stylar end of the ovary. This bending continues as growth progresses, so that the ovule is half anatropous at maturity (figs. 25-28).

MEGASPOROGENESIS.—A hypodermal cell at the apex of the ovule becomes differentiated as the archesporial cell (fig. 15). This cell divides to form a primary parietal cell and a primary sporogenous cell (fig. 16). ROY did not observe such a division in *Pisum sativum* and regards it as an exception for this group. The primary sporogenous cell (fig. 17), now the megaspore mother cell, enlarges during the stages leading to diakinesis, and the cytoplasm is finely vacuo-



late. It was noted in several instances that there may be two or even three sporogenous cells within an ovule (fig. 18).



FIGS. 15-28.—Megasporogenesis: Fig. 15, archesporial cell;  $\times 425$ . Fig. 16, primary parietal and primary sporogenous cell;  $\times 425$ . Fig. 17, young ovule with sporogenous cell showing early stage in development of integuments;  $\times 425$ . Fig. 18, portion of nucellus showing two primary sporogenous cells;  $\times 425$ . Fig. 19, diakinesis; seven pairs of chromosomes;  $\times 1250$ . Fig. 20, interkinesis;  $\times 425$ . Fig. 21, linear row of spores;  $\times 1000$ . Figs. 22-24, stages in development of megagametophyte: fig. 22, two-nucleate stage; fig. 23, four-nucleate stage; fig. 24, mature megagametophyte;  $\times 825$ ,  $825$ ,  $450$ . Figs. 25-27, development of integuments and formation of half anatropous ovule;  $\times 85$ ,  $85$ ,  $45$ . Fig. 28, longitudinal section through young ovary showing position of ovules;  $\times 5$ .

Seven pairs of chromosomes are present at diakinesis (fig. 19). Two cells are formed following the first meiotic division (fig. 20).

Both nuclei divide during the second meiotic division to form two cells each. The result of these divisions produces usually four megaspores (fig. 21). The chalazal cell which becomes the functional megaspore enlarges greatly and becomes elongated.

The megaspore nucleus divides mitotically to form a two-nucleate megagametophyte. A large vacuole forms between the two nuclei, which are found at each end of the megaspore (fig. 22). Further divisions of these nuclei lead to development of a four (fig. 23) and later of an eight-nucleate megagametophyte. Three of these nuclei remain at the chalazal end of the megagametophyte, develop cell walls, and become irregularly ovoid antipodal cells. Three nuclei remain at the micropylar end and after cell wall formation become differentiated into the egg cell and the two synergids. The egg is pear-shaped and lies with the smaller end toward the micropyle. The egg may be recognized by the nature of the cytoplasm, which is finely vacuolate at the basal region of the cell and coarsely vacuolate at its tip end. The large nucleus lies in the denser cytoplasm at the base of the cell. The synergids are irregular in shape and the cytoplasm is very dense and finely vacuolate. The small, almost abortive nuclei lie in the mid regions of the synergids. The two remaining nuclei, which become the polar nuclei, remain within the old megagametophyte wall and do not unite until the time of fertilization. The mature eight-nucleate, seven-celled, elongated and curved megagametophyte consists of three small uninucleate ovoid antipodals imbedded in the nucellus, a large binucleate primary endosperm cell, two irregularly shaped synergids, and a pear-shaped egg cell (fig. 24). It was observed occasionally that two megagametophytes were present in an ovule, one larger than the other.

### Summary

1. Each theca of the anther of *Pisum sativum* contains many microspore mother cells.
2. Cell plates are formed on the cytoplasmic strands midway between the nuclei of the tetrad.
3. The microspore cell divides to form a two-celled pollen grain.
4. The tube nucleus disintegrates before germination of the pollen grain.

5. The generative cell divides within the pollen tube to form two male gamete cells.
6. Each ovule usually contains one primary sporogenous cell; two or three cells have been observed in some instances.
7. An apical hypodermal archesporial cell divides to form a primary sporogenous cell and a primary parietal cell.
8. The primary sporogenous cells function as megaspore mother cells.
9. Usually four megaspores are formed as a result of the meiotic divisions.
10. The chalazal megaspore develops into an eight-nucleate, seven-celled megagametophyte; the other megaspores disintegrate.
11. One megagametophyte is usually found in an ovule; occasionally two are present.
12. The mature ovule is half anatropous.
13. Two integuments are present.
14. The haploid chromosome number is seven.

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# MACROSPOROGENESIS AND EMBRYO DEVELOPMENT IN *ULMUS FULVA*

RUTH I. WALKER

(WITH TWENTY- EIGHT FIGURES)

## Introduction

SCHNARF (3, 5) has summarized the various types of development of the megagametophyte in the angiosperms. In the *Adoxa* type, until recently referred to as the *Lilium* type, the macrospore mother cell develops directly into the megagametophyte, without the formation of a linear row of macrospores. SHATTUCK (6) has reported that this type occurs in the majority of cases in *Ulmus americana*. STENAR (7) described a modification of the *Adoxa* type in *Gagea lutea*, in which the macrospore mother cell undergoes two divisions, forming a linear row of four macrospore nuclei. All four nuclei may divide again, forming a typical eight-nucleate megagametophyte; or only the three nuclei nearest the micropyle may divide and the chalazal nucleus degenerates. DAHLGREN (1) has reported that all four macrospore nuclei enter into the development of the megagametophyte of *Armeria* and *Statice*. A similar condition in its formation has been found by HAUPT (2) in *Plumbago capensis* (in disagreement with DAHLGREN), but variations occur in the number and the position of the antipodals and in the number of nuclei forming the endosperm.

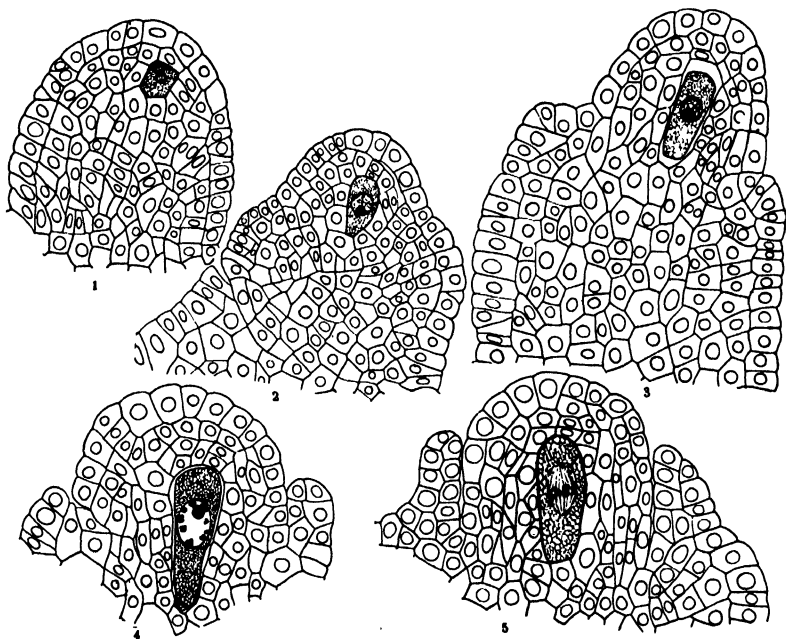
## Material and methods

Flower buds of *Ulmus fulva* Michx. were collected during the spring of 1934, and in the spring, summer, and autumn of 1936 from a native tree growing in the suburbs of Milwaukee. This material was fixed in Karpechenko's modification of Navashin's, Flemming's medium, and Carnoy's acetic-alcohol-chloroform solutions. Since the buds of *Ulmus fulva* are very hairy and float on the surface of aqueous fixatives, they were first submerged in Carnoy's solution for a few seconds before being transferred to the Flemming's medium or Karpechenko's mixtures. The fixed material was imbedded in paraffin. Sections were cut from 8 to 20  $\mu$  in thickness and were

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stained with safranin and crystal violet or with Heidenhain's iron-alum haematoxylin. A counter stain of fast green was used with the latter.

All drawings were made with a camera lucida at table level. Spencer compensating oculars, and Spencer 16 mm. N.A. 0.25, and 4 mm. N.A. 0.66 achromatic objectives and 1.5 mm. 1.25 N.A. achromatic oil immersion objective were used.



FIGS. 1-5.—Development of ovule: Fig. 1, nucellus of young ovule showing hypodermal archesporial cell. Fig. 2, ovule as it appears in the autumn showing origin of inner integument. Fig. 3, ovule the following spring; macrospore mother cell in early prophase. Fig. 4, macrospore mother cell, diakinesis. Fig. 5, heterotypic metaphase  $\times 380$ .

## Observations

### OVULE DEVELOPMENT

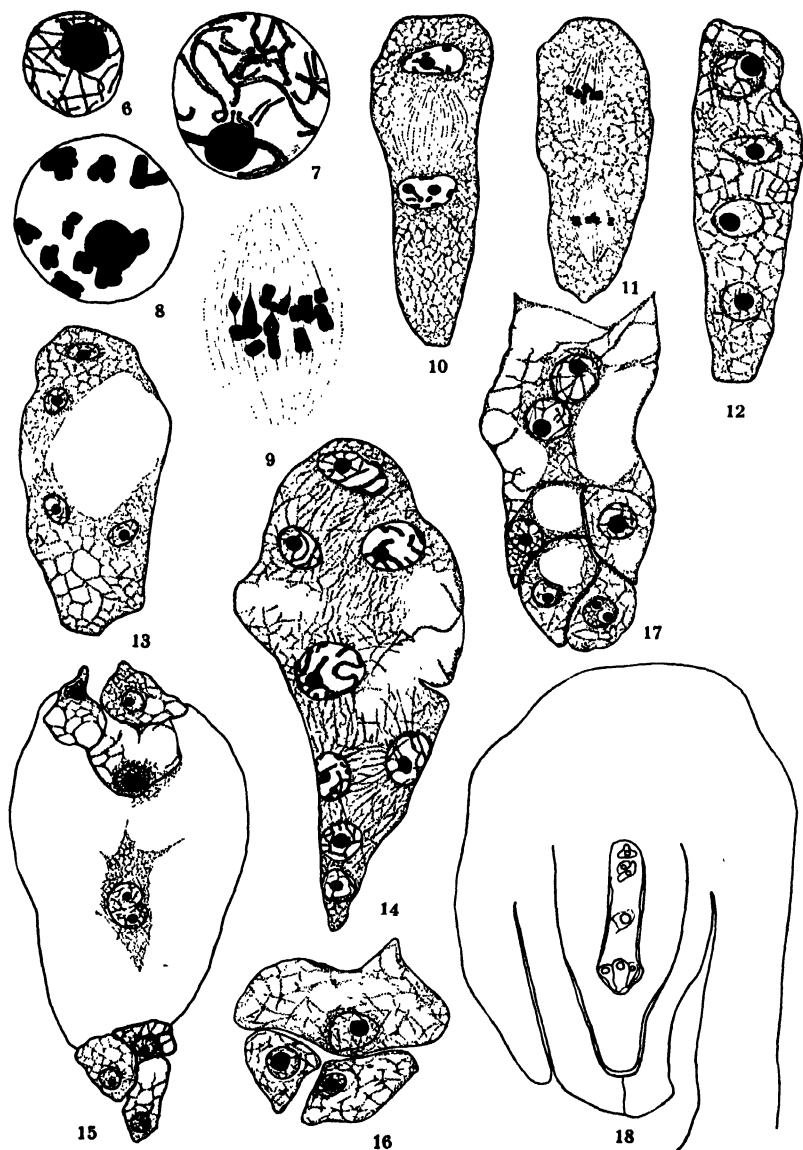
A single anatropous ovule develops in each ovary. The nucellus arises as a meristematic mass of cells on the inner surface of the ovary wall (fig. 1). The young ovule bends toward the stylar end of the ovary. The inner integument begins its development in the

autumn as an outgrowth from the epidermal layer, at a level just below that of the primary sporogenous cell (fig. 2), while the outer integument begins development the following spring, about the time of the heterotypic division in the macrospore mother cell (fig. 5). This integument arises just below the inner integument. Both integuments continue to grow and reach a level almost even with the apex of the nucellus by the time the four macrospore nuclei are formed. When the macrogametophyte is mature, the integuments inclose the nucellus, leaving only the micropyle (fig. 18).

#### MACROGAMETOPHYTE DEVELOPMENT

Early in August, a single hypodermal cell (fig. 1) is differentiated at the apex of the nucellus as an archesporial cell. This cell functions as the macrospore mother cell and is easily recognized by its greater size and deeper staining properties. It grows considerably in length (fig. 2). When it is about three times as long as broad, growth ceases until the following spring (fig. 3).

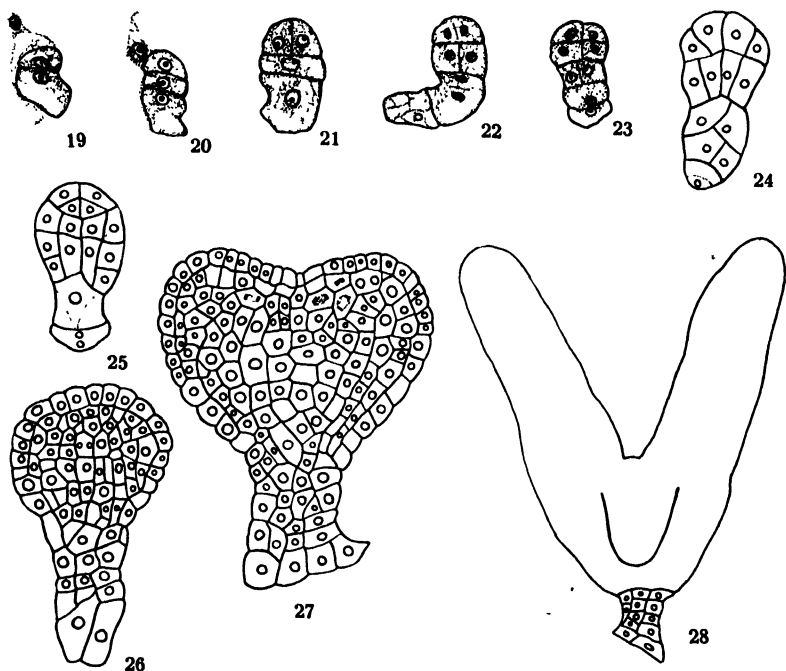
The nucleus of the macrospore mother cell contains a single large nucleolus, imbedded in the chromatic network (fig. 6). The latter condenses to form dense threads (fig. 7) which shorten, thicken, and appear as pairs of chromosomes (fig. 8). Figure 9 shows fifteen pairs of these at the equatorial plate of heterotypic division. The two daughter nuclei (fig. 10) resulting from the heterotypic division shortly undergo the homoeotypic division (fig. 11), and four nuclei appear in a linear row (fig. 12). The spindle fibers disappear, and the cytoplasm becomes uniformly vacuolate. Many instances were seen in which the vacuoles tended to coalesce gradually, forming a large central vacuole (fig. 13). At no time is there any indication of a cell plate. The formation of four macrospore nuclei without the intervention of cell walls agrees with SHATTUCK'S (6) observations in *Ulmus americana*. The macrogametophyte grows and the four nuclei divide (fig. 14). The large central vacuole previously present has disappeared at the eight-nucleate stage. Three of the eight nuclei remain at each end of the macrogametophyte; the two polar nuclei migrate toward the center of the cell. These come in contact and fuse before fertilization to form the primary endosperm nucleus (fig. 15).



FIGS. 6-18.—Stages in development of macrogametophyte: Fig. 6, macrospore mother cell in autumn, early prophase;  $\times 2000$ . Fig. 7, the following spring;  $\times 2000$ . Fig. 8, diakinesis;  $\times 2000$ . Fig. 9, heterotypic division, equatorial plate;  $\times 2000$ . Fig. 10, two-nucleate macrogametophyte;  $\times 950$ . Fig. 11, homoeotypic division of macrospore nuclei;  $\times 950$ . Fig. 12, four-nucleate macrogametophyte;  $\times 950$ . Fig. 13, same showing large central vacuole;  $\times 950$ . Fig. 14, eight-nucleate macrogametophyte;  $\times 950$ . Fig. 15, mature seven-celled macrogametophyte;  $\times 500$ . Fig. 16, transverse section of synergid and egg;  $\times 950$ . Fig. 17, chalazal end of macrogametophyte showing four antipodals;  $\times 950$ . Fig. 18, mature ovule with beaklike nucellus;  $\times 125$ .



The egg enlarges and assumes an ovoid form. At maturity, large vacuoles occupy its apical end; the nucleus is located in the basal portion, surrounded by dense cytoplasm (fig. 15). Adjacent to the egg are two pear-shaped synergids. A cross section through the micropylar end of the macrogametophyte (fig. 16) shows the relative



FIGS. 19-28.—Development of embryo: Fig. 19, two-celled proembryo. Fig. 20, three-celled proembryo showing apical and basal cells. Fig. 21, vertical division of apical cell. Fig. 22, transverse division of apical cell occurring before vertical division. Fig. 23, eight-celled embryo. Fig. 24, sixteen-celled embryo; multicellular suspensor. Figs. 25, 26, 27, further development of embryo and suspensor. Fig. 28, embryo showing cotyledons, epicotyl, hypocotyl, and suspensor. Figs. 19-27,  $\times 290$ ; fig. 28,  $\times 105$ .

position of the egg and synergids. The antipodal cells vary in shape, but tend to become elongated as the macrogametophyte matures. They do not usually disintegrate before fertilization, as reported by SHATTUCK. Occasionally four antipodals are present (fig. 17), and in one instance a macrogametophyte with twelve nuclei was observed—a condition frequently found by SHATTUCK. While the

macrogametophyte is enlarging, the cells of the nucellus at the micropylar end increase in number, forming a beaklike structure (fig. 18) which extends into the micropylar region. The cells of the beak are larger and stain more darkly than the surrounding cells.

#### EMBRYO DEVELOPMENT

The zygote divides transversely to form a two-celled embryo, consisting of a small apical cell and a larger basal cell (fig. 19). A transverse division of the apical cell results in a tier of three cells (fig. 20). Usually a vertical division occurs in the apical cell before the subapical one divides transversely and vertically (fig. 21). Exceptions to this rule were observed as shown in figure 22. After the division of the subapical cell the proembryo consists of five cells, of which the two at the apical end develop into the embryo proper and the remaining three into the suspensor. Periclinal divisions (figs. 24, 25) occur in the embryo proper, cutting off the dermatogen. In consequence of further divisions, the embryo becomes a spherical mass of cells and later heart-shaped (fig. 27). The suspensor becomes multicellular. Two leaflike cotyledons are differentiated at the apex of the embryo. The epicotyl arises between the cotyledons at the apex of the embryo. The basal portion of the embryo develops into the hypocotyl (fig. 28).

#### Summary

1. The ovule of *Ulmus fulva* is anatropous, consisting of two integuments and a massive nucellus which becomes beaklike at maturity.
2. The archesporial cell functions as a macrospore mother cell.
3. The macrospore mother cell nucleus undergoes meiosis, forming four macrospore nuclei in a linear row.
4. No cell plates or walls are formed between the macrospore nuclei.
5. A typical eight-nucleate macrogametophyte is formed as a result of one further nuclear division.
6. The zygote, by transverse divisions, forms a row of three cells. The apical cell of this row develops into the embryo. The two basal cells form the suspensor.

The writer is indebted to Professor C. E. ALLEN for helpful suggestions and kindly criticism during the course of this study.

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# HORMONES AND ROOT FORMATION

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## Introduction

Beginning with SACHS (7) in 1880, investigators have explained root formation on cuttings on the basis of the accumulation of special root forming substances near the basal cut surface. Success in obtaining an active root forming substance, however, was first achieved by WENT (12) in 1929. He found that substances which were active in inducing roots on *Acalypha* cuttings diffused from leaves of *Acalypha* and *Carica papaya*, when placed with their petioles in water. Later THIMANN and KOEFLI (9) showed that synthetic indole(3)acetic acid, a growth substance, was active in promoting root formation on pea cuttings.

Since these initial researches, the promotion of root formation by indole(3)acetic acid and other synthetic growth substances has been observed by other workers (3, 5, 10). Experiments recently reported by the writer (4) indicate that indoleacetic acid controls the movement of other substances within the plant which are necessary for root formation. Lemon cuttings treated at the base with a strong solution of indoleacetic acid developed a great number of roots at the base. If, however, the treated bases were cut off immediately after treatment and the cuttings treated again, no more roots were initiated than on untreated cuttings. This suggests that other substances necessary for root formation accumulated at the base under the influence of the original indoleacetic acid treatment, and were removed when that portion was cut off. Additional experiments have been conducted and a study has been made of the relation of the amount and distribution of auxin, and of the influence of the presence or absence of leaves on root formation.

<sup>1</sup> The studies here reported were made in the biological laboratories of the California Institute of Technology at Pasadena, California, and represent part of a thesis presented to the faculty of the Graduate School of the California Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The writer is indebted to Professor F. W. WENT for many helpful suggestions and criticisms.

### Material and methods

Eureka lemon and Delicious apple stem cuttings were used as experimental plants. The cuttings, unless otherwise noted, were 12 cm. long, from mature terminal growth, and had six to eight buds 1.5–2 cm. apart. Two full sized leaves were left at the apex. The basal cut was made just below a bud. Ten to twenty cuttings were used in each experiment, and in all cases the standard error of the mean for root counts is given.

Water solutions of synthetic indole(3)acetic acid (Merck) were used in all experiments. The basal ends in some cases and the apical ends in others were placed in the test solutions to the depth of 1 cm., and were left in nearly saturated atmosphere during the treating period (20 to 40 hours as noted specifically for each experiment). On removal from the test solution, the cuttings were rinsed with tap water and the morphological bases were inserted about 3 cm. deep in sand in a sash-covered propagating frame with bottom heat thermostatically controlled at 30° C.

### CHLOROFORM EXTRACTION TECHNIQUE

By certain modifications in THIMANN'S (8) chloroform extraction method it was possible to extract and measure quantitatively the auxin in treated lemon cuttings. The procedure adopted in this investigation was as follows: 2.5 gm. of freshly scraped bark was immersed in a mixture of 45 cc. of Merck's reagent chloroform and 5 cc of 1 N HCL. After 20 hours the bark was filtered off and the chloroform layer separated from the filtrate. The chloroform was distilled off, leaving an oily residue; 2.5 ml. of water was added to this residue and this was heated for one hour over a water bath at about 75° C. The water was poured off, and several dilutions were made of it for the auxin test. An agar plate (8 × 10.7 × 1.5 mm.) was added to each dilution and was left for one hour. Finally the agar plate was cut into twelve equal blocks and tested for auxin by the standard *Avena* technique (14).

The average curvature obtained for the twelve blocks was multiplied by the amount of the dilution of the 2.5 cc. water extract (containing auxin from a 2.5 gm. sample) and this value was in turn

divided by 2.5 to obtain "units auxin per gram sample." These units are equivalent to about 160 "*Avena* Einheit" (AE) of KOGL and HAAGEN SMIT (6). A control 10-unit stock solution of indoleacetic acid was tested on each day of the experiment in order to determine the variation in the sensitivity of the *Avena* test plant. The experimental determination for auxin in the samples under test was corrected accordingly.

In all probability the growth substance extracted from cuttings treated with indoleacetic acid is largely that acid. In this paper, however, the more general name, auxin, is used because a small amount of the native growth hormone, probably auxin a, was also extracted from untreated cuttings (table 1).

In several instances a second extraction of the bark was tested. The results were as follows:

Sample no.	First extraction	Second extraction
1	560 units	42 units
2	664	58
3	110	6

Since the amount of auxin in the second extraction was in all instances less than 10 per cent of that of the first, the procedure of only one extraction was adopted as it was desirable to have as simple process as possible. The results should be considered as relative only and not as representing the absolute amount of auxin in the sample.

## Results and discussion

### RELATION OF AMOUNT AND DISTRIBUTION OF AUXIN IN CUTTINGS TO ROOT FORMATION

LEMON CUTTINGS TREATED AT BASE.—Table 1 shows the amount of auxin obtainable from the bark at the apex and base of cuttings at different times after treatment at the base with 0.001, 0.005, and 0.02 per cent indoleacetic acid solutions. Ten leafless lemon cuttings were used in each extraction, apex extractions being made from 3 cm. of bark at the extreme apex and base extractions from 3 cm. of bark at the extreme base. Any bark in excess of 2.5 gm. fresh weight in each sample was discarded.

TABLE 1

DISTRIBUTION OF AUXIN IN BARK OF LEAFLESS LEMON CUTTINGS AT DIFFERENT TIMES AFTER TREATMENT AT BASE FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

PERCENTAGE CON- CENTRATION OF INDOLEACETIC ACID	TIME IN RESPECT TO 20 HOUR TREATMENT	AUXIN IN UNITS PER GRAM BARK	
		APEX	BASE
0.02.....	Just before	1	1
	Just after	3	680
	1 day after	1	30
	3 days after	0	4
	10 days after	0	0
	20 days after	0	4
0.005.....	Just before	1	1
	Just after	0	34
	1 day after	0	18
	3 days after	0	.....
	10 days after	0	1
	20 days after	0	0
0.001.....	Just before	1	1
	Just after	3	8
	1 day after	0	1
	3 days after	0	0
	10 days after	0	0
	20 days after	0	0
Tap water.....	Just before	1	1
	Just after	0	0
	1 day after	0	0
	3 days after	0	0
	10 days after	0	0
	20 days after	0	0

TABLE 2

ROOT FORMATION ON LEAFLESS AND LEAFY LEMON CUTTINGS TREATED AT BASE FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

PERCENTAGE CON- CENTRATION OF INDOLE- ACETIC ACID	AVERAGE NUMBER OF ROOTS PER CUTTING AFTER THREE WEEKS*	
	LEAFY	LEAFLESS
0.02.....	28.6 ± 3.8	11.0 ± 1.0
0.005.....	1.7 ± 0.6	1.4 ± 0.3
0.001.....	0.8 ± 0.4	0.6 ± 0.1
Tap water.....	0.9 ± 0.3	0.5 ± 0.2

\* Includes roots plus root primordia. Twenty cuttings in each experiment.

TABLE 3

DISTRIBUTION OF AUXIN IN BARK OF LEAFLESS LEMON CUTTINGS AT DIFFERENT TIMES AFTER TREATMENT AT APEX FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

PERCENTAGE CONCENTRATION OF INDOLEACETIC ACID SOLUTION	TIME IN RESPECT TO 20 HOUR TREATMENT	AUXIN IN UNITS PER GRAM BARK				
		MARCH EXPERIMENT		APRIL EXPERIMENT		
		APEX	BASE	APEX	MIDDLE	BASE
0.02 .....	Just before	*	2	4	3	4
	Just after	790	8	1280	†	3
	1 day after	72	20	60	4	3
	3 days after	17	3	11	0	1
	10 days after	3	0	9	0	0
	20 days after	*	*	5	0	0
0.005 .....	Just before	*	2	4	3	4
	Just after	41	6	72	8	10
	1 day after	8	2	8	16	6
	3 days after	2	*	2	0	0
	10 days after	1	1	*	0	0
	20 days after	*	*	0	0	0
0.001 .....	Just before	*	2	4	3	4
	Just after	12	2	8	3	4
	1 day after	3	5	2	12	2
	3 days after	2	2	0	0	0
	10 days after	2	0	0	0	0
	20 days after	*	*	0	0	0
Tap water. . .	Just before	*	2	4	3	4
	Just after	2	2	2	1	1
	1 day after	*	*	1	0	0
	3 days after	*	*	1	1	1
	10 days after	*	*	0	0	0
	20 days after	*	*	0	0	0

\* No determination made.

† Below 20 units.

TABLE 4

ROOT FORMATION ON LEAFLESS LEMON CUTTINGS TREATED AT APEX FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

PERCENTAGE CONCENTRATION OF INDOLEACETIC ACID	AVERAGE NUMBER OF ROOTS PER CUTTING AFTER THREE WEEKS*	
	AVERAGE NUMBER	INCREASE OVER TAP WATER CONTROLS
0.02 .....	1.8 ± 0.5	1.3 ± 0.5
0.005 .....	0.6 ± 0.2	0.1 ± 0.2
0.001 .....	0.5 ± 0.1	0
Tap water .....	0.5 ± 0.2	.....

\* Includes roots plus root primordia. Twenty cuttings in each experiment.



The bark of the treated base of the cuttings showed large amounts of auxin immediately after treatment, indicating that solutions of the strengths tested are actually absorbed by the cutting. The amount, however, was by no means proportional to the concentration of the solution. The stronger solutions were absorbed much more readily than the weaker ones. The 0.02 per cent solution was only four times as strong as the 0.005 per cent solution, yet about twenty times as much auxin was recovered from the former. Likewise over eighty times as much was extracted from the cuttings treated with 0.02 per cent as from the 0.001 per cent cuttings, while the difference in concentration of solution was of the order of one to twenty.

The relatively large amounts of auxin occurring in the base of the cuttings immediately after treatment did not persist long, about 95 per cent disappearing during the first day in the case of the 0.02 per cent treatment. The absence of any appreciable amount of auxin in the apex at any time indicates that there was no upward movement to the apex. A few extractions were made of the wood, which showed, just as with the bark samples, very little auxin in any part of the cutting other than that immersed in the solutions. The fact that all extractions were made on leafless cuttings almost completely eliminated transpiration as a factor affecting upward transport in the xylem. As yet no extensive data have been obtained on distribution of auxin in leafy cuttings.

Root counts on both leafy and leafless cuttings treated with these same solutions are given in table 2. It is seen that with both leafless and leafy cuttings, only the 0.02 per cent solution was effective in root formation, the 0.001 and 0.005 per cent solutions being no more effective than tap water. Since more auxin was found in the base of cuttings treated with 0.001 and 0.005 per cent solutions than in cuttings treated with tap water, it appears that some factor other than auxin was limiting root formation. In the case of treatments with the 0.02 per cent solution, this other factor was apparently no longer limiting since a large number of roots were initiated. One possible explanation for this response is that the strong indoleacetic acid solution mobilized the other factor.

LEMON CUTTINGS TREATED AT APEX.—When the cuttings were treated at the apex with the 0.001 and 0.005 per cent solutions there

was apparently a downward transport of auxin, as indicated by the recovery of moderate amounts of auxin in the base as compared with very little in the base of control samples (table 3). This accumulation of auxin in the base was greater after treatment with the 0.005 than with the 0.001 per cent solution. In the apical treatments with the 0.02 per cent solution, a moderate amount of auxin was recovered from the base samples in one experiment (more than for the 0.005 per cent solution) but very little in the other experiment (less than for the 0.005 per cent solution). The amounts recovered in both cases were extremely small as compared with the amount occurring in the apex. Apparently applying high concentrations to the apex brings about some abnormal condition in the tissue at the point of application, for after the high level of auxin is quickly reduced, the remaining small amount is retained in the apex for a period of at least 20 days even when other regions of the cutting are devoid of auxin in measurable amounts. Since this fact was not observed in connection with the 0.005 and 0.001 per cent solutions, it is suspected that the normal polar transport is upset by the use of high concentrations, and that in this case transport is influenced by the condition of the cutting at the time of the treatment.

It is seen from table 4 that very few roots were formed at the base on any of the cuttings treated at the apex. The failure of the 0.02 per cent solution to give the pronounced response obtained by basal treatments is accounted for by its limited downward transport in lemon cuttings. In some instances treatment at the apex (with 0.02 per cent or higher concentration) caused root formation both at the apex and base. This condition occurred only occasionally, however, but would perhaps have been more pronounced had temperature and moisture conditions been as favorable for root development at the apex as at the base. On the basis of relative auxin content, one would expect a greater number of roots to appear at the apex than at the base.

APPLE CUTTINGS TREATED AT BASE.—So far all attempts to root Delicious apple cuttings with indoleacetic acid treatments have failed. Cuttings of various kinds and conditions taken at every month of the year were used, and not one root was observed. The question then arose as to whether apple cuttings failed to absorb the acid or contained some mechanism whereby the substance was de-

stroyed on being absorbed. In an attempt to solve this problem, apple and lemon cuttings were treated in the usual manner with a 0.02 per cent indoleacetic acid solution for 20 hours, and auxin extractions were made of the bark of both the lemon and apple: (1) immediately after the treatment, (2) the next day, and (3) three days after the treatment. The results given in table 5 show that

TABLE 5

COMPARISON OF AMOUNTS OF AUXIN RECOVERABLE FROM BARK OF LEAFLESS LEMON AND APPLE CUTTINGS AT DIFFERENT TIMES AFTER TREATMENT AT BASE WITH 0.02 PER CENT SOLUTION OF INDOLEACETIC ACID FOR 20 HOURS

MONTH TREATED	TIME AFTER TREATMENT	LEMON		APPLE	
		AUXIN IN UNITS PER GRAM OF BARK		AUXIN IN UNITS PER GRAM OF BARK	
		APEX	BASE	APEX	BASE
January.....	7 hours	12	89	18	92
March.....	{ Immediately	4	680	4	800
	{ 1 day	1	20	2	232
	{ 3 days	0	4	1	7

there is very little difference in the amounts of auxin recovered from the lemon and apple cuttings. Failure of the apple to respond to indoleacetic acid therefore appears to be due, not to excessive destruction of the substance or to a failure of the cuttings to absorb it, but to causes other than a lack of the acid. One possible explanation is that apple cuttings are lacking in certain internal substances which are present in lemon cuttings and which are necessary for root formation. Evidence that internal substances are involved in the rooting of lemon cuttings by means of indoleacetic acid is presented in the following experiments.

#### EFFECT ON ROOT FORMATION OF CUTTING OFF THE TREATED BASE AND TREATING THE NEW BASE

The possible role of the strong solution (0.02 per cent) of indoleacetic acid in causing root formation on lemon cuttings is seen from a study of the results given in table 6. Cuttings treated at the base

with a 0.02 per cent solution for 20 hours produced  $11.7 \pm 1.0$  more roots per cutting than did the tap water controls. Cutting off the treated base destroyed the effect of the treatment, and treating the new base for 20 hours with 0.02 per cent solution caused only  $5.8 \pm 1.2$  more roots than on the controls, or about half as many as found on treated cuttings without subsequent treatment. Excising the treated base likewise destroyed the effect of a 40 hour treatment

TABLE 6  
EFFECT ON ROOT FORMATION OF EXCISING THE TREATED  
BASE AND TREATING THE NEW BASE

EXPERIMENT NO	INITIAL TREATMENT	SUBSEQUENT TREATMENT	AVERAGE NO. OF ROOTS PER CUTTING AFTER THREE WEEKS*
A.....	0.02% indoleacetic acid for 20 hours	None	$12.6 \pm 1.8$
B.....	"	Base cut off	$1.1 \pm 0.3$
C.....	"	Base cut off and new base treated with 0.02% indoleacetic acid for 20 hours	$6.8 \pm 0.7$
D.....	Tap water for 20 hours	None	$0.9 \pm 0.3$
E.....	0.02% indoleacetic acid for 40 hours	None	$17.5 \pm 1.9$
F.....	"	Base cut off	$0.4 \pm 1.5$
G.....	"	Base cut off and new base treated with 0.02% indoleacetic acid for 20 hours	$1.1 \pm 0.3$

\* Twenty cuttings in each experiment.

with 0.02 per cent solution, but treating the new base for 20 hours with 0.02 per cent solution gave no more roots than when not treated. Thus the number of roots obtained after cutting off a treated base and re-treating the new base depends on the length of the initial treating period. Cuttings with an initial treatment of 20 hours gave  $5.8 \pm 1.2$  more roots than controls, while those with an initial treatment of 40 hours gave no more roots than the controls. In order to insure equal amounts of stored foods and other substances, the final length of the cuttings in all experiments was made the same. Cuttings in experiments B, C, F, and G (table 6) were

made about 3 cm. longer in the beginning to allow for the removal of 3 cm. of the stem at the base after the initial treatment.

These results are in agreement with earlier work by the writer (4), and are explained by assuming that the indoleacetic acid applied at the base in strong solution causes the downward transport of a substance (or substances) called rhizocaline,<sup>2</sup> which is necessary for root formation. These substances apparently accumulate in the base of the cutting under the influence of indoleacetic acid, and cutting off the treated base removes them from the cutting. The fact that there were 12.6 roots per cutting for a regular 20 hour treatment and 6.8 roots when the 20 hour treated base was cut off and the cutting re-treated 20 hours suggests that about twice as much rhizocaline moved to the base during the first 20 hours of treatment as during the second 20 hours. Also the failure to obtain roots by re-treating 40 hour-treated cuttings indicates that perhaps the rhizocaline movement to the base in this particular experiment was complete after 40 hours.

A similar conclusion regarding the role of an internal factor in root formation was reached by WENT (13) from experiments with pea cuttings. He found that, if a cutting were divided into a number of sections and each treated with a high concentration of auxin, the sum of the numbers of root primordia formed was about the same as on an intact cutting so treated. This suggests that the number of primordia is determined by internal substances, which limit root production even though auxin is in excess. The bulk of the primordia were on sections some distance from the apex; therefore they must have contained more of the internal substances. When auxin was applied to the apex of the uppermost one-eighth of a cutting, only about seven primordia were formed at the top, but when applied to the apex of the intact cutting, thirty primordia were formed at the top. The auxin therefore appears to have mobilized some other substance from the lower parts of the cutting.

Although the results of experiments on lemon suggest that an important function of indoleacetic acid in root formation is to control

<sup>2</sup> The term rhizocaline was first used by BOUILLENNE and WENT (2) in referring to naturally occurring root-forming substances. The same meaning is implied in the use of the term in this paper.

the transport of rhizocaline, it does not preclude the possibility that the acid may, for instance, also react with or activate rhizocaline to cause differentiation of the root meristem. Some recent experiments by WENT (unpublished data) with pea cuttings suggest that indoleacetic acid does play a dual role in root formation by first mobilizing rhizocaline and then reacting with it.

#### INFLUENCE OF LEAVES ON ROOT DEVELOPMENT

Since the results just presented suggest that indoleacetic acid caused the downward transport of rhizocaline, it was thought that

TABLE 7  
EFFECT OF LEAVES ON ROOT FORMATION ON LEMON CUTTINGS  
TREATED AT BASE FOR 20 HOURS WITH 0.02 PER CENT  
INDOLEACETIC ACID SOLUTION

LOT	TIME LEAVES REMOVED	AVERAGE NUMBER ROOTS PER CUTTING AFTER THREE WEEKS*		
		ROOTS	ROOT PRIMORDIA	TOTAL
H.....	Before	4.3 ± 0.9	6.8 ± 1.2	11.1 ± 1.4
I.....	Immediately after treatment	5.3 ± 1.3	23.3 ± 3.9	28.6 ± 3.8
J.....	Twenty hours after treatment	7.1 ± 1.2	17.0 ± 2.1	24.1 ± 2.2
K.....	One week after treatment	11.3 ± 1.0	22.5 ± 3.6	33.8 ± 3.5
L.....	Leaves not removed	13.0 ± 0.6	10.8 ± 1.9	23.8 ± 2.1

\* Ten cuttings in each experiment.

possibly this substance came primarily from the leaves. If the acid causes the rapid transport of this substance from the leaves to the base of the cutting, removing the leaves shortly after treatment should have no effect on the number of roots formed, as the rhizocaline will already have moved to the base. The light intensity in the propagating frames was so low that it is doubtful whether much assimilation occurred in leafy cuttings after being placed in the frame.

With these considerations in mind, an experiment was conducted whereby leaves were removed from lemon cuttings before treatment and at various times after treatment. The results are given in table 7. It is noted that the number of fully developed roots increased

with the time that the leaves were left on the cuttings. When root primordia were included, however, the total number of roots initiated for all lots which had the leaves on at the time of treatment (I to L inclusive) was found to be roughly the same. It is true that there is a wide range in the values for these four lots, which no doubt is due to the difficulties involved in making an accurate determination of the root primordia; but they show no definite trend in relation to the time the leaves were removed, and all show a significantly greater number of roots initiated than lot H which had no leaves at the time of treatment.

These results indicate that leaves do furnish substances necessary for root formation, because all cuttings with leaves intact during the treatment showed a significantly greater number of roots than cuttings with no leaves. These results are substantiated by similar results from many other experiments with lemon cuttings. Those roots which are initiated on treated leafless cuttings are accounted for by the trapping of rhizocaline in the cutting at the time of taking it. It seems likely that in the intact plant rhizocaline is synthesized in the leaves, and moves basipetally in the twigs and branches to the roots. At the time of taking the cutting, therefore, the stem tissue as well as the leaves will contain rhizocaline.

The fact that the presence of leaves after the 20 hour treatment with indoleacetic acid had no further influence on the number of roots initiated substantiates the view that the acid causes the rapid downward movement of rhizocaline. The observation, however, that the presence of leaves after the treatment does influence the number of root primordia to elongate indicates that in this case we are concerned with substances coming from the leaves necessary for the outgrowth of root primordia. This substance (or substances) is apparently transported slowly to the base, because removing the leaves after one week gave marked reduction in the number of developed roots. Also it has been repeatedly observed that there is a significant increase in the number of developed roots on treated leafy cuttings between three and six weeks. This would be expected on the basis of the data in table 7, which show an average of  $10.8 \pm 1.9$  root primordia per cutting still present in the base of treated leafy lemon cuttings after three weeks. On the other hand there was never a

significant increase in number of visible roots between three and six weeks and leafless cuttings, although histological examinations showed a great number of root primordia present. It appears that the leaves on lemon cuttings furnish substances necessary for further growth of root primordia, and that these substances are different from root-forming substances.

Experiments similar to the preceding have been conducted on chrysanthemum cuttings and the results are presented in table 8. In these experiments the cuttings were not examined for root primordia so it is not possible to segregate the separate actions of root-forming and root-elongating substances. Nevertheless the data show the same relation between the time leaves were removed and the number of visible roots as in the case of lemon cuttings.

It is noted from table 8 that the indoleacetic acid treatment caused an injury to the base of cuttings without leaves while no injury was observed on cuttings with leaves. With lemon also it has frequently been noticed that leafy cuttings will tolerate a stronger treatment than leafless cuttings. At present no explanation can be offered for these observations.

#### ATTEMPTS TO EXTRACT RHIZOCALINE FROM LEAVES

Chloroform extracts of leaves of a number of different plants have failed to show any response other than that of promoting growth of the *Avena* coleoptile, and therefore probably contained the naturally occurring growth hormone, auxin a. The growth hormone apparently is not rhizocaline, however, because Delicious apple leaves and bark were found to contain as much of it as the lemon. Thus it seems likely that factors other than auxin are involved.

A second attempt to extract rhizocaline was made by placing petioles of cut-off leaves of several different plants in a strong solution of indoleacetic acid, the idea being that the acid would mobilize the rhizocaline from the leaf. The results of one experiment with lemon leaves showed that the diffusate into the acid appeared to increase slightly the root-forming activity of the indoleacetic acid solution. More experiments must be conducted to determine whether the difference is real. All types of leaves are apparently not adapted to this technique because the diffusate from *Lantana* leaves definitely



destroyed the effectiveness of the indoleacetic acid solution, while fig leaf diffusates reduced the activity of the acid to half its normal value. Enzymes set free from the cells at the cut surface of the petiole may have destroyed the acid in a manner similar to that ob-

TABLE 8  
EFFECT OF LEAVES ON ROOTING OF UNTREATED AND TREATED  
CHRYSANTHEMUM CUTTINGS\*

VARIETY	INDOLEACETIC ACID TREATMENT	TIME LEAVES REMOVED	TIME IN SAND (WEEKS)	AVERAGE NUMBER ROOTS PER CUTTING†	TREATED BASE INJURED
Meta Bergen	None	First day after treat- ment	2	0	
		Third day after treat- ment	2	4.5 ± 1.0	
		Not removed	3	11.3 ± 1.6	
		First day after treat- ment	3	6.3 ± 1.3	
		Third day after treat- ment	3	6.9 ± 0.8	
		Not removed		10.2 ± 1.5	
Willard's Bronze....	0.01% for 18 hours	Before treatment	3	2.2 ± 0.9	+
		Third day after treat- ment	3	15.5 ± 1.2	—
		Not removed	3	26.3 ± 1.6	—
	Tap water for 18 hours	Before treatment	3	0.5 ± 0.3	—
		Not removed	3	0.3 ± 0.05	—

\* Cuttings placed in sash-covered propagating frame without bottom heat.

† Ten cuttings in each experiment.

served by VAN OVERBEEK (11) for sections of bases of corn mesocotyl.

Conclusive evidence of the existence of rhizocaline and other naturally occurring substances, as postulated in this paper, awaits actual extraction. Further work is now in progress. At the same time various combinations of sugars, amino acids, and vitamins are being tested in connection with and following the indoleacetic acid treatment. It is suspected that one of the substances necessary for the outgrowth of the root primordia is vitamin B<sub>1</sub>, since this substance has been found by BONNER (1) to be necessary for the growth of excised pea roots.

### Summary

1. Portions of cuttings, some of which had been treated at the apex and some at the base with indoleacetic acid, showed large amounts of auxin in the bark immediately after treatment, but about 90 per cent of the auxin disappeared during the first day.

2. Very little auxin was recovered from the bark at the apex of cuttings which had been treated at the base with 0.001, 0.005, and 0.02 per cent solutions of indoleacetic acid.

3. Moderate amounts of auxin were recovered from the base when the cuttings were treated at the apex with these solutions; however, with the 0.02 per cent solution the amount recovered at the base was small as compared with that at the apex.

4. The 0.02 per cent solution applied at the base of lemon cuttings was the only treatment of those tested that gave significant increase in root formation on lemon cuttings over controls.

5. There was little difference in the amount of auxin recovered from treated apple and lemon cuttings, yet the apple cuttings did not form roots. It is assumed that apple cuttings are lacking in certain internal substances necessary for root formation.

6. Experiments involving removal of the treated base and treating of the new base indicate that the action of indoleacetic acid in root formation is primarily the mobilization of naturally occurring root-forming substances.

7. Evidence is presented which indicates that leaves of lemon cuttings supply a substance necessary for the differentiation of the root primordia and another for the outgrowth of these primordia. The former appears to be transported rapidly to the base under the influence of indoleacetic acid while the latter is transported slowly to the base.

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## CORRELATIVE EFFECTS OF ENVIRONMENTAL FACTORS ON PHOTOPERIODISM<sup>1</sup>

KARL C. HAMNER

In 1920 GARNER and ALLARD (7, 8) announced the results of experiments and observations on the responses of plants to alternating periods of light and darkness, or variations in the length of day and night. The term photoperiod was adopted to designate this periodic alternation as an environmental factor, and the term photoperiodism to designate the responses on the part of the plant.

What constitutes a long day or short day may be arbitrarily fixed by an investigator, but in dealing with the response of flowering the concept of GARNER seems particularly pertinent. He states (11): "For those plants which are more sensitive to the length-of-day factor, there exists a fairly definite critical light period which constitutes the dividing line between day lengths favorable to flowering and fruiting and those tending to produce a purely vegetative type of activity. In the short-day group flowering is initiated by day lengths shorter than the critical, and in the long-day group flowering is initiated by day lengths in excess of the critical. Commonly among annuals and herbaceous perennials the alternative vegetative stage of the short-day type is characterized by indeterminate elongation of the axis, while a prominent feature of the vegetative stage in the long-day type is embodied in a leaf-rosette form of growth without significant stem elongation. To determine the classification of a given plant it will usually suffice to observe the nature of the response to a day length less than and to one greater than the critical light period. The essential characteristic of the less sensitive or indeterminate group of plants is that they possess no clearly defined critical light period."

It is important to keep in mind the indeterminate group, since a

<sup>1</sup> Invitation paper read at the joint meeting of Section G, American Association for the Advancement of Science, the Botanical Society of America, American Phytopathological Society, American Society of Plant Physiologists, Mycological Society of America, at Indianapolis, December 28, 1937.

great many attempts have been made to apply directly the results of experiments on that group to an analysis of the results of experiments with the other two groups. As an example, the endeavors to apply the chemical results found associated with flowering and fruiting of the tomato—an intermediate or day-neutral plant—have failed to account for the initiation of flower primordia, although the data apply very well to fruit setting and fruit development.

Although in the literature most emphasis has been placed upon the flowering responses of plants to photoperiod, photoperiodism involves any response of a plant to changes in photoperiod. In their earliest papers, GARNER and ALLARD pointed out that vegetative extension, tuberization, and other responses are definitely similar. They state (9), "The available data indicate that there is an optimal light period for tuber formation just as there are optimal periods for stem elongation and for sexual reproduction. The highest proportionate production of tubers occurs with a light period which admits of only very limited stem growth. The absolute maximum production of tubers, however, is obtained with an intermediate light period." . . . "Within the range of duration of light exposures which will admit of alternative forms of expression there are in most cases certain exposures favorable only to sexual reproduction and other exposures favorable only to vegetative activity. That is, there are more or less definite optima in light exposures for the two alternative forms of expression. The question arises as to the response of the plant to light exposures intermediate between those specifically favorable to the two types of activity. In reality the effects of these intermediate exposures are important and are both quantitative and qualitative in character."

Much confusion has arisen in discussions involving a quantitative delimitation of the vegetative, flowering, fruiting, or reproductive condition in relation to environmental factors or internal condition, especially chemical composition, because of the failure of specific designation of the material investigated. Much of what has been said concerning the effect of photoperiod on the reproduction of angiosperms is not easy to integrate, especially since the angiosperms are a group in which it is particularly difficult to set a limit within which plants are distinctly vegetative or distinctly reproductive. If,

as is often done, gametic differentiation and gametic union are considered the final criteria for determining sexual reproduction, then much of what has been written about blooming, fruiting, and so forth has no particular significance as sexual reproduction, but must be considered only as a correlated condition.

In attempting an integration of several factors affecting photoperiodism or determining the role of photoperiod as one of several factors influencing plant behavior and development, it is essential first of all that the vegetative states and the flowering and reproductive states be thought of, not as antagonistic functions, but as correlated conditions which constitute a range of qualitative expression. An apple tree of moderate size may produce one blossom cluster, or a hundred, or a hundred thousand clusters. In any of these cases the tree may be considered as being in flower. Qualitatively the three situations are alike if blooming of the tree is made the basis of classification, but the quantitative differences are very great and so too are the physiological conditions within the tree as a whole.

It may be difficult qualitatively to differentiate between a vegetative and a reproductive plant. The transition from floral leaf to vegetative leaf may be very gradual. It would easily be possible to arrange a closely graded series of structures which all agree are vegetative, through those which are in close association with the sexually reproductive structures, to the sexually reproductive structures themselves, that is, the gametes. It is better therefore to speak of the flowering or non-flowering condition, rather than of the reproductive condition, unless such reproduction actually involves gametic differentiation. That such reference has a very real point will be brought out later in relation to the failure of pollen grains or gametophytes to come to maturity under certain conditions of photoperiod and mineral nutrient supply, although apparently fully developed flowers may be produced. There is need for precise records showing the influence of photoperiod upon (1) the initiation of flower primordia as contrasted with (2) its influence upon the development of flowers, (3) its influence upon the development of fruits, and (4) its influence upon the development of seeds.

In this connection it is important to reconsider GARNER's concept

of the critical period previously given. It is possible for many plants to differentiate floral primordia at day lengths both longer and shorter than the critical period, but the subsequent development of these varies greatly. An example is the Biloxi soy bean, a short-day plant (7, 9). On plants maintained at or just below the critical period, approximately a 13-hour day, a condition approaching ever-blooming is attained, the primordia develop into flowers in which pollination takes place, and fruits and seeds develop. If the plants are maintained at day lengths appreciably shorter than the critical, at exposures of 8 hours or less, the primordia may develop into flowers which are small, often minute; but it is only seldom that full sized pods are formed, and few seeds are produced. This condition is associated with marked suppression of vegetative extension of the plant as a whole as compared with plants which have differentiated floral primordia and have continued to be maintained at the critical period. On the contrary, on plants which are held for a few days at the critical period or slightly below it, until flower primordia have been initiated, and then transferred to a photoperiod longer than the critical, at least some of the primordia may continue to develop into flowers which are very much larger than those on plants maintained at or below the critical period. The carpels or fruits of these may fail to develop, or develop very slowly, and often contain no seeds. Associated with this condition is a much greater vegetative extension of the entire plant, leaves, length of internodes, and diameter of the stems. Under similar conditions of photoperiods greater than the critical, the flower buds of chrysanthemum may abort completely and the plant as a whole be definitely vegetative. In the case of *Cosmos* (2) some of the floral primordia continue development, but the flower heads and individual flowers exhibit wide ranges in type, the corollas of some of the outer ray flowers being green and leaflike. Some of the flower heads may appear as close clusters of small green leaves. Just as in the former cases mentioned, these conditions are associated with a pronounced vegetative state of the plant as a whole, the most vegetative condition being associated with complete failure of differentiation of floral primordia. As previously indicated, it is important that at least four phases, initiation of flower primordia, development of flowers, of fruits, and of seeds,

be kept in mind as distinct segments on a graded scale, the margins of such segments intergraded but their midportions clearly recognizable. Chemical studies (19) have shown certain distinct correlations with the several types of development after flower primordia have been initiated, but of the substances so far determined none has been found which accounts for the initiation of such primordia. After such initiation the developmental behavior of photoperiodically sensitive plants closely parallels that of the non-sensitive, indeterminate, or day-neutral tomato.

Because of the normal shifting of the length of day in a natural environment in most latitudes, and because of the preponderance of so-called switch-over experiments under controlled environments, it is important to integrate the effect which one environment, to which a plant has been exposed, exercises when the plant as a whole, or some of its parts (10), is placed under another environment. This period of exposure to a given environment whose effects are carried over and continue to be manifest under a changed environment may well be referred to as an induction period (16). Thus the term photoperiodic induction, or after-effect, would mean the carry-over effect exhibited when a plant is transferred from one photoperiod to another. Some short-day plants will initiate flower buds if given as few as three short days and subsequently grown on long day. Thermo-induction, the carry-over effect of temperature as an environmental factor, has long been recognized but more recently has been re-emphasized (24, 6, 18). Similarly one might well consider a chemo-induction period, as is well illustrated by plants when grown under varying planes of nutrient supply. As a specific example, some plants once brought to the fruiting condition with a relatively low plane of nitrogen supply will continue to fruit at a plane so high that, if initially supplied to them, fruiting would not have taken place. This terminology is largely a matter of breaking up into somewhat more specific verbiage the general term of KIDD and WEST, physiological predetermination (13), and would include much that is now listed under the term yarovization or vernalization. Despite the terminology, relatively little is known concerning the precise method of operation of these inductive influences in relation to subsequent developmental patterns or behavior. The existence



of such possible carry-over effects should be recognized. Failure to take them into account has led to some wholly inadequate and erroneous interpretations of supposed immediate effects of the environment, which effects were in reality the expression of a reaction to the environment to which the plants had been exposed previously as well as the immediate one.

Since the discovery of the effects of photoperiod upon flowering and fruitfulness, many efforts have been made to correlate length of day and nutrition. Most of these attempts have been designed to see whether changes in photoperiod affect the carbohydrate-nitrogen relations of the plant in sufficient degree to explain the initiation of the flowering state. In general, this has been found not to be the case. Most short-day plants growing on a long day cannot be induced to form flowers by wide variations in the amount of nitrogen supply or by conditions which favor or inhibit carbohydrate synthesis. Even though the light intensity during any given photoperiod be greatly reduced, as is experienced in the winter months as compared with those of summer, the time required for floral initiation of any given variety of plant is not greatly changed, although the number of floral primordia initiated may be considerably fewer. Also the subsequent development of such primordia is dependent upon the intensity of illumination during the photoperiod. Very commonly floral primordia initiated during a long or a short photoperiod of low intensity will fail to continue to develop into flowers or fruit, whereas if the light intensity is high during all or a considerable portion of the photoperiod, they will continue to develop.

Even in the early work on the carbohydrate-nitrogen relationships associated with blooming and fruiting of the tomato (15), emphasis was placed upon their effect upon fruitfulness rather than on the initiation of flower primordia. It was stated that "the conditions for the initiation of floral primordia and even blooming are probably different from those accompanying fruit setting. The greatest number of flowers are produced neither by conditions favoring highest vegetation nor by conditions markedly suppressing vegetation." Unfortunately in many subsequent attempts to apply those findings to other plants, these distinctions have been largely overlooked.

Lacking as yet a determination of a specific substance or substances, on the basis of which the initiation of floral primordia can be accounted for, it is possible to say only that in many plants the initiation of the flowering condition is determined by the day length. Evidence of a correlation between chemical composition and type and character of subsequent development of such primordia after they have been initiated is far more clear and direct (19).

It has been found that nitrogen deficiency induced the earing of barley plants, a long-day type, growing on a 9-hour day, whereas with an abundance of nitrogen the plants failed to produce ears (3). A 9-hour day would be considered below the critical day length of barley, and hence, if photoperiod were the only factor to be considered in blooming, the plants should not have blossomed. There has been at least one report (1) that nitrogen deficiency could affect the time of flowering of a short-day plant growing on a short day and a long-day plant growing on a long day, but the reports demonstrating that the plane of nitrogen nutrition may be substituted for changes in photoperiod in inducing flowering in long and short-day plants have been few.

It remains to be shown whether changes can be effected if plants are grown at their critical photoperiod. It is certain that short-day plants may be affected when grown on day lengths longer than the critical after an induction period; the longer the length of day above the critical period, the more striking the effect. Many of the experiments which have dealt with this problem have involved, consciously or otherwise, plants which have been subjected to an induction period consisting of a few short days at the critical period or below it. During this period flower primordia were initiated and the plants then transferred to day lengths longer than the critical period. Under such circumstances the plants generally flower and often produce fruits, the extent of flowering being dependent upon the length of day above the critical period, the greater this lengthening the more vegetative the plant and the less the amount of flowering or seed production. As has been pointed out, if short-day plants are grown at day lengths appreciably below the critical period, not only the extent of flowering but also their vegetativeness is concurrently decreased. At photoperiods slightly greater than the critical period,

flowering and fruiting may be increased in association with an increased condition of vegetativeness; but if the length of the photoperiod is greatly increased, flowering and fruiting are decreased along with an increase in vegetativeness. Experience has shown that the addition of relatively large increments of nitrogen to plants below the critical period has little or no effect on the behavior of the plant, but above the critical period the addition of nitrogen does increase the degree of vegetativeness and the total net weight of the plants (20). Within certain ranges the number of flowers and fruits produced may also be augmented.

Because of the decided differences in behavior of plants at photoperiods above and below the critical period in relation to their response to nitrogen supply, it would be worth while to attempt some more specific evaluation of the critical period in terms of physiological response or chemical behavior. Thus controlled photoperiods offer one of the most valuable tools for investigating the whole problem of metabolism, not only in relation to flowering and fruiting but in relation to the entire range of vegetative response, either as such alone or in association with the differentiation of floral primordia, and their possible consequent development. What is now termed the critical period may well mark a rather definite point of change in physiological behavior, and should therefore furnish a valuable clue for the selection of materials on which to base physiological and chemical analyses,

Although the general qualitative response of the plant seems to be conditioned primarily by photoperiod, in general the quantitative range of expression of this response is influenced by the plane of nitrogen supply. For example, *Xanthium pennsylvanicum*, the cocklebur, will flower in a short time if grown on a short day and remain vegetative on a long day. Restriction of the nitrogen supply will not induce flowering in the plants being subjected to the long day, nor will an abundant supply inhibit flowering of those on the short day. Subsequent to a photo-induction period, however, the plants on a long day with abundant nitrogen grow more rapidly and produce more flowers and fruits which come to maturity at a slower rate than if the plane of nitrogen supply is low. The same general type of response is manifested by the plants maintained on a short

day, except that all the plants mature more rapidly than those on the long day. The greatest numbers of fruits per plant are produced under conditions of the short day with a comparatively high nitrogen supply.

*Salvia splendens* (20) when grown on a 17-hour day with an abundance of nitrogen will produce abundant vegetative growth but no flowers. If a plant which has been growing for some time under such conditions is transferred to a day length of about 14 hours, it will produce flowers. The number of flowers produced under such conditions is greatly influenced by the supply of nitrogen, however, plants receiving little or no nitrogen developing many more flowers than plants receiving a plentiful supply. It may be that the 14-hour day is near the critical period for *Salvia*, and because of this fact the supply of nitrogen produces a marked effect.

Work has been done relating to a more critical dissection of these results in terms of chemical composition and in terms of cytological detail. In some plants, changes in photoperiod affect the capacity of the plant to assimilate nitrogen (20). Thus certain short-day plants growing on a photoperiod shorter than the critical may take up large quantities of nitrates which are not assimilated but accumulate in the plants as nitrates. Possible changes in the carbohydrate-nitrogen relations have been correlated more directly with the development of flowers and fruits than with the differentiation of flower primordia (19). Cytologically (12) it has been shown that in the tomato, a day-neutral form, plants deficient in carbohydrates and having a great abundance of nitrogen were unfruitful, not primarily because flowers failed to differentiate but because no functional pollen grains developed. If plants of the cocklebur are given a photo-induction period of several days and are then divided into four lots, short-day low nitrogen supply, short-day high nitrogen, long-day low nitrogen, and long-day high nitrogen, all four lots will flower but each lot shows a characteristic development.

The short-day low nitrogen plants develop relatively few flowers, either staminate or pistillate, but the anthers contain the highest percentage of fertile pollen grains of any of the lots.

The short-day high nitrogen plants develop more staminate

flowers than those on low nitrogen, but the stamens contain a higher percentage of sterile pollen grains.

The long-day plants on low nitrogen develop fewer flowers, both staminate and pistillate, than similar plants on high nitrogen, but the flowers mature more rapidly. The number of defective pollen grains is about the same as the short-day high nitrogen plants.

The long-day plants with high nitrogen develop the greatest number of flowers and the greatest length of time is required for the fruits to reach maturity. The number of defective pollen grains is about the same as in the other lots, except the one on short day and low nitrogen, which it will be recalled had by far the greatest number of fertile grains.

While the number of reports showing that nutrition can substitute for or nullify the effects of photoperiod are decidedly limited, the claims that temperature can do this are many. Plants such as stocks (21), beet (6), and celery (24) tend to be strictly vegetative if grown continuously at relatively high temperatures, but at relatively low temperatures they flower readily and develop seeds. A combination of these two conditions may be made if plants are grown for a time at a high temperature, then placed for a few days at a relatively low temperature which serves as a thermo-induction period, and then are returned to a high temperature. Under such circumstances, either floral primordia are initiated during the induction period or the character of the plant is so changed that blooming, and frequently fruit setting and seed formation, take place at the higher temperature. Depending upon the length of the induction period and the temperatures maintained after the induction period, a more or less intergraded series of responses from strict vegetativeness to a combination of vegetativeness, flowering, and fruiting in all degrees may be manifested. To give a specific example, stocks remain indefinitely in the vegetative stage when exposed continuously to temperatures around 70° F. If, however, a plant is given a thermo-induction period of a few days at temperatures approximating 50° F. and is then returned to the higher temperature, in a short time flowers and fruits are produced. This performance is characteristic of many plants, after they have undergone thermo-induction. There are also types of plants, such as lettuce (25), which undergo thermo-

induction at relatively high temperatures. The short exposure at a high temperature will exert a carry-over effect causing the plants to flower when subsequently grown at a low temperature. ✓

It has been demonstrated that many plants which exhibit striking photoperiodism show little photoperiodic sensitivity after a thermo-induction period, usually flowering more rapidly the longer the light period. The fact that a plant may be thermoperiodic need not mean that it may not also be sensitive to photoperiod. The critical work that has been done on the beet (6) illustrates this point. If beets are grown at temperatures between 50° and 60° F., all the plants produce flowers in a short time. If they are grown continuously at temperatures fluctuating between 60° and 70° F., they will produce flowers provided they are exposed to a long day and not if they are exposed to a short day. If they are grown continuously at 70° F. or above, they will not produce flowers at any day length. Some varieties of soy beans, beet, and *Rudbeckia*, grown under precisely controlled conditions of temperature and light (23), have different quantitative responses to photoperiod when grown at different temperatures. Thus an increase in temperature somewhat decreases the range of critical day length for Biloxi soy beans, a short-day plant, and *Rudbeckia*, a long-day plant. With the sugar beet, also a long-day plant, an increase in temperature increases the range of critical day length.

Much confusion has arisen in discussions attempting to establish a direct correlation between the behavior of temperature sensitive plants and their chemical composition, because of failure to differentiate between qualitative and quantitative behavior. Although many plants require a certain set of conditions to bring about induction of the flowering condition, it seems generally true that, after induction has been completed, flowering and fruiting may be accelerated by a different set of conditions. Thus most plants which have undergone thermo-induction at a relatively low temperature will flower and set fruit much sooner if the temperature is then raised following the induction period (17). The sugar beet, as produced in southern Utah, following the cold induction period of the winter season, will flower abundantly with increased day length and temperatures which may range well above 70°. Increased increments of

nitrogen supply given following such an induction period do not inhibit blooming but result in greatly increasing the numbers of flowers and seeds produced (22). It has been suggested (17) that sexual reproduction in winter and spring wheats is not dependent upon a critical temperature or a critical light period, but the time at which it occurs is influenced greatly by both these environmental factors. Winter wheats develop flowers most rapidly if they grow under conditions of increasing day length and increasing temperature, after initial stages of short-day length and low temperature. Instead of considering winter wheats as short-day, long-day, low temperature, high temperature plants, their behavior can be harmonized with others just mentioned by regarding them as plants which respond to induction periods of low temperature and short days. Their quantitative yields would then be somewhat proportional to the degree of higher temperature, longer light period, and increased nutrient supply to which they were subjected after the induction period. Spring wheats apparently do not require or at least do not respond to the same type of induction periods.

Several investigators have suggested that during photoperiodic induction there is manufactured in the leaves of plants a flower-forming hormone, tentatively called florigen, which is transmitted to the meristems causing them to differentiate flower primordia. Spinach (14), a long-day plant, will not respond to long day if only a small portion of the plant near the growing point receives long day and the rest of the plant is left on a short day. The growing point will respond only if an appreciable leaf area is included in the treatment. Investigators in Europe (4, 5) have performed a variety of experiments which also indicate that the leaves are the organs which are sensitive to the photoperiodic stimulus. Thus if entire plants of *Chrysanthemum indicum* are grown on a long day they remain in the vegetative condition. If, however, alternate leaves on a plant grown under general conditions of a long day are exposed to a short day, all the axillary buds begin to form flower primordia, but only those in the axils of the treated leaves finally develop into flowers. Similarly, if one single leaf of such a plant is exposed to a short day, the bud or branch in the axil of that leaf will form flower

primordia, provided such an axillary branch does not have any of its leaves expanded.

✓ The results of such experiments and the general failure to find any marked correlation between the amounts of carbohydrates, nitrogenous complexes, or similar compounds associated with initiation of flower primordia, have caused a number of investigators to postulate the presence of very minute quantities of some more recondite compound or compounds as being directly responsible for their differentiation. These are assumed to be present in the meristems, and may thus become activated or destroyed, as the case may be, by changes in photoperiod, thermoperiod, or some other environmental change. Others assume these are manufactured in the leaves, stems, or other organs of the plant and are transferred to the meristem. Most investigators prefer to withhold judgment until substances which have been isolated from the plant and synthesized in the laboratory can be applied, and the response in question secured. As yet such specific substances have not been extracted, identified, or synthesized; and even if they were now available, the method of their operation, their influence on the metabolism of the plant, and histological and structural changes following their application would still remain to be determined. What may be the relation between application of acetylene to pineapple plants (26) and the prompt differentiation of flower primordia and fruit development, which ensues in treated plants as compared with non-treated plants which differentiate floral primordia many weeks and even months later, is worthy of critical experimentation.

If substances can be found to which other plants respond as the pineapple responds to application of acetylene, experiments could be performed throwing much more light on photoperiodism. There may be many such substances, some acting under one set of environments, others under another, and in all possibility as many interactions as are postulated for the various growth promoting substances and hormones now so searchingly under investigation. Here would be another field open to the study of correlative factors and influences, which would after all be largely a change in phraseology rather than in point of view, in any endeavor to correlate environmental



factors and their interaction. This must be done if the present situation concerning factors influencing vegetative development, floral differentiation, fruit and seed development is to be clarified.

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# GROWTH PATTERNS OF PLANTS DEVELOPED FROM IMMATURE EMBRYOS IN ARTIFICIAL CULTURE<sup>1</sup>

H. B. TUKEY

(WITH NINETEEN FIGURES)

## Introduction

In the course of culturing immature embryos of deciduous fruits excised from the fruit at various stages in development (23), a definite and characteristic type of growth or growth pattern has been observed for the plants. Instead of completing the usual embryonic development from the zygote to the mature seed, as occurs on the parent plant, they have developed in culture into plantlets which exhibit a definite conformation or growth pattern apparently related to the age of each embryo when excised.

Although there are no direct references in the literature to growth patterns, as such, from immature embryos in artificial culture, most workers have called attention to some phase or another of unusual growths. HANNIG (9) experienced difficulty with very young embryos of *Cochlearia* and *Raphanus* and reported several anomalous growth forms. Other workers have had similar experience with very small or very young embryos, including STINGL (17) with embryos of *Secale*, *Triticum*, *Hordeum*, and *Avena*; DIETRICH (3) with *Impomea* and *Allihea*; WHITE (27) with *Portulaca*; TUKEY (18, 23) with *Prunus* and *Pyrus*; and LA RUE (11) with *Lactuca*, *Taraxacum*, *Chrysanthemum*, and *Zea*. In all these instances immature embryos began to grow immediately without first going through the usual embryonic stages.

On the other hand LAIBACH (12), working with partially developed embryos of *Linum*, found that they did not germinate immediately when excised; but if they were placed in vials on cotton wadding watered by a 10-15 per cent solution of cane sugar, they developed to normal mature embryos which then germinated. Likewise KNUDSON (10) grew orchid embryos unremoved from the seed

<sup>1</sup> Journal Paper no. 254 of the New York State Agricultural Experiment Station. The writer is indebted to the National Research Council for a grant to carry on this work.

in culture and eventually these germinated and developed into normal plants. WERKMEISTER (26) found that if poorly developed seeds of *Iris* were placed on nutrient 10 per cent agar agar with Pfeffer's solution and 0.5 per cent glucose, they gradually imbibed water and developed so that later they could be germinated. All such plants eventually developed normally.

Mature or nearly mature seed has also produced unusual types of growth, but of a different nature from those from very young embryos. FLEMION (4) found that mature but non-after-ripened seeds of *Rhodotypos kerrioides* developed into plants with a dwarfish appearance, and with short, stocky hypocotyls and internodes, and small, dark green leaves. She later (5) reported a similar type of growth for non-after-ripened embryos of peach, apple, and hawthorn. DAVIDSON (1), in culturing immature peach embryos, described all plants raised in culture as abnormal and dwarfish, having small, wrinkled, and peculiarly curled leaves. VON VEH (25) found that seedlings of apple, pear, quince, plum, and cherry raised from non-after-ripened embryos developed into dwarf plants. LAMMERTS (13) secured similar results with apricot, peach, cherry, rose, and camellia; and GERSHOY (6) with the violet.

### Material

Twelve varieties of sweet cherry (*Prunus avium* L.), five of sour cherry (*P. cerasus* L.), three of European plum (*P. domestica* L.), two of American plum (*P. americana* Marshall), thirty-two of peach (*P. persica* Batsch.), one of apricot (*P. armeniaca* L.), five of apple (*Malus domestica* Borkh.), and four of pear (*P. communis* L. and *P. communis* × *P. serotina*) have been used during five growing seasons, 1932 to 1936 inclusive.

Most of the material was from the varietal orchards of the Experiment Station at Geneva, New York, at which the work was carried on. Fruits for dissection were gathered fresh as needed. For comparative purposes fruits were also secured from Youngstown in Niagara County, New York,<sup>2</sup> and from Athens, Georgia.<sup>3</sup> Shipments from Youngstown arrived in 18 hours in good condition for dissec-

<sup>2</sup> Supplied through the courtesy of Dr. W. S. REED.

<sup>3</sup> Supplied through the courtesy of Dr. H. J. HARROLD.

tion. Shipments from Athens, Georgia, consisted of fruiting branches carefully packed in damp moss wrapped in wax paper, the cut ends of the branches being set in a damp sponge of moss. Packed in this way, twenty shipments of five varieties of peaches (covering a range of 49 days in season of fruit ripening), made at weekly intervals from full bloom to fruit ripening, were received in fresh condition within 36 hours of gathering. Material from all three sources was equally viable.

Embryos were dissected at intervals during the growing season, from April to October, covering the stage from zygote to seed maturity. In all, more than 20,000 individual cultures have been made.

### Methods

Two procedures of culture have been employed: (A) using a disinfectant; (B) using aseptic conditions. The method involving a disinfectant (18) consisted in gathering the fruit, washing it, and opening it in the laboratory under approximately sterile conditions. The embryos were then submerged for 5 minutes in calcium hypochlorite solution prepared according to WILSON's formula (31). It contains almost exactly 20,000 p.p.m. of chlorine and has proved most satisfactory. Washing with sterile water following the treatment has proved of no advantage and increases the chances for contamination.

When desirable to avoid the presence of disinfectant in contact with the material or the media, fruits were washed in a 0.1 per cent solution of mercuric chloride, and all operations, including transfer, were done in a transfer room. In some instances, with very young embryos 0.16 mm. in length, dissections were made in broth of the same concentration as the nutrient media upon which the cultures were subsequently grown. That is, in making up the media, several test tubes of solution were set aside and sterilized in which to dissect material later to be cultured in the same media. Such cultures were made on culture micro slides, in hanging drops, and in yeast culture chambers on micro slides, in addition to the larger containers described in following paragraphs. With careful attention to technique, complete freedom from contamination may be secured without recourse to disinfecting agents.

In transferring the embryos from the calcium hypochlorite solution, regular bacteriological technique proved most satisfactory. The small amount of calcium hypochlorite solution introduced with the transferred material had no apparent deleterious effect, while the film of solution aids in preventing contamination during transfer.

For culture chambers, square screw-cap bottles with aluminum metal caps proved superior to other containers tried, such as petri dishes, covered watch glasses, and test tubes. When cultures are maintained for 8 to 24 months, there is less likelihood for the media to dry than with cotton plugged test tubes, and less opportunity for contamination as in the growing of an organism through a cotton plug. Tests with and without caps screwed tight have shown no disadvantage from tight closure, and have the advantage of less contamination over a period of months. For large embryos, as the peach, 1 ounce bottles are good; for smaller embryos, such as the cherry, pear, and apple, the  $\frac{1}{2}$  ounce size is adequate.

For a considerable portion of the studies a culture solution has been used which consists of 0.6 per cent agar, 0.5 per cent glucose, and 0.15 per cent of the following salt mixture, designated T:

SALT MIXTURE T	
10 gm. KCl	} Salts ground and mixed, and 1.5 gm. added to 6.5 gm. of agar and 5 gm. of glucose in 1 liter of water.
2.5 gm. CaSO <sub>4</sub>	
2.5 gm. MgSO <sub>4</sub>	
2.5 gm. Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	
2.5 gm. FePO <sub>4</sub>	
2 gm. KNO <sub>3</sub>	

Since the medium has a pH of about 5.5, such a low concentration of agar should be either chilled in a refrigerator or otherwise rapidly cooled following autoclaving.

The salt may be kept ground and mixed in a stoppered bottle and used over a period of months as needed. There has seemed neither advantage nor disadvantage in filtering to remove any cloudiness.

In filling the bottles with medium, care has been exercised to place it in the bottom of the bottle by means of a pipette and otherwise to keep the sides and mouth of the bottle free from any medium. Sterilization has been at 15 pounds pressure for 20 minutes.

Unless otherwise indicated, cultures have been grown in daylight

in room temperature (*ca.* 18° C.). Using the technique described, peach embryos have been developed into small plants and maintained in a slowly growing condition in 1 ounce bottles for 17 months.

When plants have developed sufficient root and top growth, they may be shaken from the bottles, together with the agar medium, and planted in sterile quartz sand in 1½ inch pots until the plants are sufficiently large to shift to soil in larger pots. Young plants should be carefully shaded and gradually hardened.

AGAR CONCENTRATION.—Tests have been made with agar ranging from 0.5 to 10 per cent. WERKMEISTER (26) has reported good results with *Iris* seed cultured on 10 per cent agar, but not only has it been difficult to prepare so stiff a gel, but the behavior of similar embryos has been variable. Embryos which develop into plants soon suffer from lack of moisture. Both 1 and 2 per cent concentrations have proved useful, but best results have been secured with 0.6 per cent. At this concentration the agar is sufficiently stiff to support the embryo on the surface, yet availability of water does not prove a limiting factor. Lower concentrations, as 0.5 per cent, have proved difficult to use because of the ease with which they liquefy. La RUE's data, using a 0.75 per cent agar, agree closely with these results.

SALT CONCENTRATION.—A wide range of salt mixtures and concentrations have been used with similar results. These include that used by ROBBINS in root cultures and by WHITE in culturing root tips (28, 29); Knop's solution at dilutions used in sand cultures; and formula T, adapted from Crone's nitrogen-free formula.

Although concentrations have been varied from one to ten times a given strength, there has been no appreciable effect so long as the concentrations were not toxic to the plant. These results are in agreement with the previous findings by DIETRICH (3), TUKEY (23), and LA RUE (11), indicating a wide range of tolerance.

pH.—The general purpose formula used in these tests as formula T has a pH of about 5.5. Because the growth of embryos may alter the pH of the media, constant pH cultures were prepared after the formulae of ZINZADZE (32). These ranged from 3.8, 5.2, 6.0, 7.2, 8.2, and 8.6. Embryos grow to small plants on these media, with no

deleterious effect other than a slightly chlorotic appearance of leaves of plants grown for 70 days at pH 8.6, and a tendency for the media at 3.8 to liquefy in time owing to acid hydrolysis. The pH of these media remained unchanged throughout the experiment as tested at completion of the growth period.

ORGANIC AND GROWTH-PROMOTING SUBSTANCES.—The addition of various sugars to the media had an appreciable effect upon development, as will be later mentioned; but the chief responses from the addition of organic compounds, from temperatures, and from different photoperiods must be left to another paper.

The following organic compounds and growth-promoting substances have produced no consistent response in the manner and at the concentrations used:

Heteroauxin\*

Indoleacetic acid 1 p.p.m.

Indoleacetic acid 1 p.p.m. and glycoll 100 p.p.m.

Indoleacetic acid 10 p.p.m.

Indoleacetic acid 0.1 p.p.m.

Indolebutyric acid 1 p.p.m.

Indolebutyric acid 1 p.p.m. and glycoll 700 p.p.m.

Indolepropionic 1 p.p.m.

Indolepropionic 10 p.p.m.

Propionic acid 1 p.p.m.

Propionic acid 10 p.p.m.

Yeast extract 200 p.p.m.

Yeast extract 10 p.p.m.

Yeast extract 10 p.p.m. and glycoll 10 p.p.m.

Glycoll 100 p.p.m.

\* Supplied through the courtesy of Dr. F. W. WENT  
and Dr. K. V. THIMANN.

### Growth of normal embryo

The developmental morphology of the embryo, seed, and fruit of the species to be studied has an important bearing upon the culture of excised embryos of deciduous fruits. Using the peach (20) as an example, growth of the carpel is in three stages: stage I, rapid development for 49 to 52 days after full bloom; stage II, retarded development from 5 to 42 days depending upon the variety; and stage III, rapid increase to maturity. During stage I the nucellus and integuments also make rapid increase, reaching maximum size by



the time stage II is reached, at which time the stony pericarp begins to form rapidly as sclerenchymatous tissue. The embryo during stage I develops slowly, in common with embryos of most angiosperms, remaining microscopic until the initiation of stage II. At that time it begins a rapid development and reaches maximum size in 12 to 28 days, depending upon the variety, followed by a period of accumulation of storage materials and internal change until maturity.

For other species the growth curves are characteristic. For the sweet cherry, stage I continues to 17 days following full bloom (19), for the sour cherry 21-22 days (21), and for the apricot 42 days (14).

Varieties of peach, cherry, and plum which produce early-ripening fruit fail to develop normally viable seed (19, 20, 21). Embryos of such varieties abort during stage II. The nucellus and integuments which have already reached maximum size collapse upon the partially developed embryo, to give the characteristic shriveled appearance of an abortive seed.

The apple and pear are similar in development but not identical. Growth of the fruit as measured by external measurements is not in the three well defined stages of the fruits of *Prunus*. The embryo grows similarly, however, in that there is delayed development following full bloom, followed in turn by a period of rapid embryo development. In the apple this rapid development begins 35 to 40 days (15, 16) after full bloom, while for the pear it is about 40 days (15).

Still another characteristic of seed of the deciduous fruits is the high energy content in the form of fat and the after-ripening period necessary for germination. Sweet cherry seed requires 16 weeks under moist conditions at 5° C. to complete after-ripening; peach seed 10 to 12 weeks; apple seed 6 to 8 weeks; and pear seed 4 to 6 weeks. These facts will be discussed more fully in relation to the results secured in culturing embryos at different stages in development.

### Results with embryos of peach

The results show a definite and characteristic relation between the stage in development at which an embryo is excised and its subsequent behavior in culture. Embryos of the Elberta peach, received

from Athens, Georgia, at weekly intervals in nineteen lots from full bloom to fruit ripening, may be taken as representative, to which other samplings and other classes of fruits will later be referred. For clarity the results with one of several culture media will be considered, namely, that consisting of the nutrient agar given on page 633 with the addition of 10 p.p.m. of dried brewers' yeast. Likewise, unless otherwise noted, procedure A has been used, in which embryos have been treated with calcium hypochlorite solution (2 per cent chlorine) for 5 minutes. The stages in development of pericarp, nucellus and integuments, endosperm, and embryo, the technique used, and the performance of the embryo are given in connection with each sampling.

1. FULL BLOOM: FRUIT IN STAGE I, 3.4 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 0.9 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—The integuments were of waxlike consistency and could be chiseled cleanly from the nucellus by means of dissecting needles sharpened to a knife edge at the points. Embryos were too small to discern, but cultures made of the entire seed dissected aseptically in broth of medium T and cultured in liquid media on micro culture slides increased in length from 0.9 to 1.4 mm. in 9 days, as compared with an increase to 3.0 mm. on the tree.

2. SIX DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 7.1 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 2.7 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—Observations were similar to those with sample 1. Since embryos were too minute to be studied in culture no records of growth could be secured, but an entire seed removed aseptically was maintained in liquid medium T for 29 days with daily changes. The nucellus and integuments elongated slightly during this period.

3. THIRTEEN DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 10.0 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 4.2 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—The embryo sac could be removed intact, all but for the micropylar region, which remained firmly affixed to the nucellus and from which it could not be removed without rupturing. Embryos were still too minute to discern in culture. Entire seeds removed aseptically increased in liquid media T from 4.2 to 6.4 mm. in length in 3 days, a rate comparable to the in-

crease on the tree. In one instance two seeds were removed from a single fruit, attached to adjacent edges of the carpel. Cultured on the same slide the one increased from 3.5 to 6.2 mm. in length in 3 days, while the other made no increase.

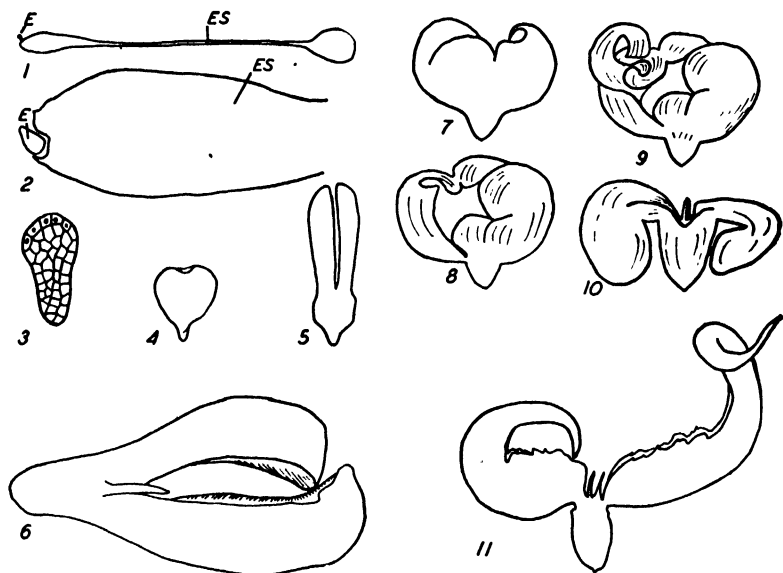
4. TWENTY DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 19.0 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 9.0 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—Using a disinfectant and culturing on agar media at this stage resulted in no increase in entire seed; and embryos were too small to be discerned.

5. TWENTY-SEVEN DAYS AFTER FULL BLOOM: FRUITS IN STAGE I, 25.8 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 10.5 MM.; CELLULAR ENDOSPERM 0.16 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—Entire seed treated with a disinfectant and placed on nutrient agar failed to increase in size, but upon dissection it was found that cellular endosperm had increased from 0.16 to 1.3 mm. in 13 days. Perhaps there may have been an increase in the size of the embryo as well, since embryo development follows endosperm development very closely (20), but the embryos were still too small to be observed in culture.

6. THIRTY-FOUR DAYS AFTER FULL BLOOM: FRUITS IN STAGE I, 34.0 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 13.6 MM.; EMBRYO SAC EXTENDING NEARLY TO CHALAZA; CELLULAR ENDOSPERM 1.6 MM. IN LENGTH; EMBRYO 0.16 MM.—The nucellus and integuments are pressed firmly against the ovary wall at this stage, and being tender and turgid are easily broken in opening the fruit. The best procedure was to split the fruit in halves along the ventral suture, the seed then being cut from the side of the carpel to which it was attached. The integuments could be stripped easily from the nucellus with needles and forceps, having passed from the softer, fleshier consistency of previous samples and not yet having reached the condition in which the integuments adhere to the nucellus and tear the nucellus with it. Furthermore, the nucellus was firm throughout and not yet digested by the endosperm.

It appears that the embryo is affixed to the micropylar end of the embryo sac, and the embryo sac in turn is affixed to the nucellus. The embryo sac could be lifted from the nucellus but not without rupturing at the micropylar end and exposing the embryo (figs. 1, 2).

It was necessary first carefully to chisel away the nucellus at the micropylar end, after which the embryo sac could be drawn out easily from the surrounding tissue. Isolated embryos failed to develop in culture following treatment with calcium hypochlorite solu-



FIGS. 1-11.—Results with peach embryos 34 days after full bloom (all drawings with camera lucida): Fig. 1, embryo sac (*es*) and embryo (*e*) dissected from immature seed 10.7 mm. long. Fig. 2, enlarged view of embryo and proximal end of embryo sac showing characteristic failure to remove embryo sac from nucellar tissue without rupturing; 1.0 mm. in length. Fig. 3, enlarged view of embryo, undifferentiated; 0.12 mm. in length. Fig. 4, differentiation of embryo in culture 10 days and increase from 0.12 mm. to 0.275 in length. Fig. 5, further differentiation of embryo 77 days in culture but abnormal; length 1.0 mm. Fig. 6, embryo cultured on agar within entire seed for 49 days, increasing from 0.32 mm. to 2.7 in length during that period. Figs. 7-10, anomalous growth of embryos in flowing media (7, 8, increases from 1.2 mm. to 3.8 in length and 4.6 mm. in width in 20 days; 9, same embryo after 166 days; 10, increases from 1.2 mm. in length to width of 2.6 mm. in 20 days). Fig. 11, 9-day development of embryo with cotyledons removed, excised from seed 61 days after full bloom; 9 mm. in breadth.

tion (2 per cent chlorine), although when cultured within the seed they differentiated and increased in size to 0.275 mm. in length in 10 days and 1.0 mm. in 77 days, as shown in figures 3-5. Treatment with calcium hypochlorite solution seemed harmful to tissues at this stage.

7. FORTY-ONE DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 40.5 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 17.1 MM.; CELLULAR ENDOSPERM 3.8 MM.; EMBRYO 0.32 MM.; COTYLEDONS DIFFERENTIATING.—Isolated embryos failed to develop in culture, although when entire seeds were cultured the embryo increased to 1.35 mm. in length in 8 days. In one instance the embryo reached 2.7 mm. in 49 days (fig. 5), with spreading and elongating cotyledons and general abnormal growth.

8. FORTY-EIGHT DAYS AFTER FULL BLOOM: FRUIT IN TRANSITION FROM STAGE I TO II, 44.5 MM. IN LENGTH; STONY PERICARP BEGINNING TO HARDEN; NUCELLUS AND INTEGUMENTS NEARLY MAXIMUM SIZE, 19.2 MM.; ENDOSPERM 8.0 MM.; EMBRYO ENTERING PERIOD OF RAPID INCREASE, 1.2 MM.; COTYLEDONS DIFFERENTIATING.—Isolated embryos treated with calcium hypochlorite solution failed to develop further in culture. Embryos dissected in broth of the medium and not treated with a disinfectant showed signs of development by a spreading of the cotyledons within 8 days, and increased from 0.9 to 3.8 mm. in length in 21 days, but failed to develop further. No chlorophyll was formed. In flowing media embryos developed into the anomalous forms shown in figures 7-10.

9. FIFTY-FIVE DAYS AFTER FULL BLOOM: FRUIT IN STAGE II, 46 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM 11.5 MM.; EMBRYOS IN PERIOD OF RAPID INCREASE, 2.0 TO 3.0 MM.—First indications of development following treatment with calcium hypochlorite solution were secured at this stage. In 48 hours after placing on agar the cotyledons had spread to 90 degrees with the central axis, while in 120 hours they had become recurved so that they touched at the tips below the hypocotyl. Chlorophyll formed in the dorsal surface tissue of the cotyledons, and the central axis of the epicotyl lengthened to 1 mm., but remained white throughout (fig. 12).

10. SIXTY-ONE DAYS AFTER FULL BLOOM: FRUIT IN STAGE II, 46 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM 16.5 MM.; EMBRYO INCREASING RAPIDLY, 5.4 MM.—Chlorophyll formed slowly, appearing first in the surface tissues of the dorsal side of the upper cotyledons. The lower cotyledon, in direct contact with the medium, then fre-

quently elongated 2 to 4 mm. and became spongy. Later the cotyledons spread at right angles to the central axis, followed by chlorophyll development on the ventral surfaces of the cotyledons. The central axis of the epicotyl lengthened to 1-2 mm., but remained white throughout, and the hypocotyl lengthened to 2-3 mm. In one instance, in which the cotyledons were excised by accident, the re-



FIG. 12.—Development of embryos excised 51 days after full bloom, showing greening and spreading of cotyledons and white epicotyl.

maining portion of the embryo developed in 19 days to the anomalous form shown in figure 11.

11. SEVENTY-THREE DAYS AFTER FULL BLOOM: FRUIT IN STAGE II, 46 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM 17.8 MM.; EMBRYO INCREASING RAPIDLY, 16.4 MM.—Chlorophyll formed rapidly in the cotyledons, which spread at right angles to the central axis, the hypocotyl elongating 2 to 4 mm. with roots developing 10 to 15 mm. in length, and occasionally to 30 mm. The central axis of the epicotyl lengthened 1 to 2 mm. and became green. It terminated in a

rosette of six to ten small white appendages 0.5 to 1 mm. in length, much resembling stipules (fig. 13*A*). The appearance of the epicotyl was that which might be produced by the failure of the internodes of a central axis to elongate and upon which only the stipules had developed and in which no chlorophyll had formed.



FIG. 13.—Typical growth patterns of peach seedlings developing from embryos excised from fruit at following ages after full bloom: *A*, 73 days; *B*, 80 days; *C*, 87 days; *D*, 94 days.

12. EIGHTY DAYS AFTER FULL BLOOM: FRUIT IN TRANSITION FROM STAGE II TO III, 48.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ALL DIGESTED; EMBRYO NEARLY MAXIMUM SIZE, 18.0 MM.—Chlorophyll formed on both dorsal and ventral surfaces of the cotyledons, which separated to 90 degrees with the central axis. The hypocotyl lengthened to 2–4 mm., frequently developing into roots 10 to 20 mm. in length, or even 30 mm. The central axis of the epicotyl lengthened to 7–15 mm. in 21 days and became green, being surmounted by a rosette of eight to twelve anomalous stipule-

like appendages 1 mm. in length, but without chlorophyll development (fig. 13*B*). Along the stem one or two green stipule-like appendages appeared in which chlorophyll developed. The general appearance was as of an elongation for an internode or two of the axis from the sample taken 73 days after full bloom, but with the internodes of the remainder of the stem still unelongated at the apex to give a rosette of white stipules.

13. EIGHTY-SEVEN DAYS AFTER FULL BLOOM: FRUIT ENTERING STAGE III, 50.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY COMPLETELY DIGESTED; EMBRYO NEARLY MAXIMUM SIZE, 18.8 MM.—Chlorophyll formed on both dorsal and ventral surfaces of the cotyledons, which spread to 90 degrees with the central axis. Roots developed on all specimens and were 10 to 15 mm. in length in 28 days. The central axis of the epicotyl lengthened to 20–22 mm., green throughout, terminating in a rosette of stipule-like appendages 1 mm. in length in which chlorophyll had not developed. True leaves developed in addition to the stipules, although occasionally such leaves were broader than is typical of peach leaves and often had crinkled margins and whitish areas along the edges. The general appearance was as of a still further elongation of the internodes of the short axis from the preceding two samples, the further development of chlorophyll in the stipule-like appendages and the development of leaves (fig. 13*C*).

14. NINETY-FOUR DAYS AFTER FULL BLOOM: FRUITS IN STAGE III, 51 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ENTIRELY DIGESTED; EMBRYO MAXIMUM SIZE, 19.8 MM.—Chlorophyll formed on both the dorsal and ventral surfaces of the cotyledons, which separated at right angles with the central axis, but the chlorophyll became less abundant than in preceding stages. Roots developed vigorously to 25–30 mm. in length in 20 days. The central axis of the epicotyl elongated to 40 mm., terminated in a rosette of small, green stipule-like appendages 1 mm. in length. Occasionally one or two stipule-like appendages appeared along the stem. They were sometimes entirely green, sometimes white, and sometimes part green and part white. Occasionally also characteristic peach leaves



formed 25 to 35 mm. in length, yet these leaves were often broad and crinkled and had whitish areas along the margins. The general appearance was as of a still further elongation of the internodes of the short stem (fig. 13D).

15. ONE HUNDRED AND THREE DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 52.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM ALMOST ENTIRELY DIGESTED; EMBRYO MAXIMUM SIZE, 19.8 MM.—Only a small amount of chlorophyll was found in the cotyledons, which spread at right angles to the central axis. Roots developed vigorously and stem length reached 40 to 45 mm., terminated in a rosette of some whitish and some green leaflike appendages 1 mm. in length. Leaves formed along the stem but were frequently broad and crinkled with whitish areas along the margins. The nodes were still relatively close together, giving the appearance of a dwarfed plant (fig. 14A).

16. ONE HUNDRED AND EIGHT DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 59.0 MM. IN LENGTH, COLORING; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM ALMOST ENTIRELY DIGESTED; EMBRYO MAXIMUM SIZE, 19.8 MM.—Roots developed vigorously and stems reached 45 to 50 mm. in length with green peachlike leaves. In 17 days the plants were transplanted from the culture bottles to soil. The leaves were often broader than typical peach leaves and the plants were dwarfish. The cotyledons developed no chlorophyll, but served as storage and nutritive organs, gradually becoming shriveled and dried (fig. 14B).

17. ONE HUNDRED AND EIGHTEEN DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 60.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ALL DIGESTED AWAY; EMBRYO MAXIMUM SIZE, 19.8 MM.—No chlorophyll found in the cotyledons at this stage. Vigorous root and shoot development occurred so that plants were transplanted to soil in 14 days. Growth was somewhat dwarfish (fig. 14C).

18. ONE HUNDRED AND TWENTY-TWO DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 63.0 MM. IN LENGTH; "HARD" RIPE; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ALL DIGESTED AWAY; EMBRYO MAXIMUM SIZE, 19.8 MM.—Plants developed with vigorous root and shoot growth, al-



FIG. 14.—Typical growth patterns of peach seedlings developing from embryos excised from fruit at following ages after full bloom: *A*, 103 days; *B*, 108 days; *C*, 118 days. *D*, future development after placing in subdued light at 45° F. for 30 days.

though somewhat dwarfish. They were transplanted to soil in 14 days. No chlorophyll formed in the cotyledons.

19. ONE HUNDRED AND TWENTY-NINE DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 65.0 MM. IN LENGTH, SOFT RIPE; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; INTEGUMENTS BROWN; ENDOSPERM NEARLY ALL DIGESTED AWAY; EMBRYO MAXIMUM SIZE, 19.8 MM.—Plants developed in 14 days which were only slightly dwarfish, with vigorous root and shoot development. They were transplanted into soil at the end of this time. Chlorophyll failed to develop and the cotyledons became somewhat shriveled, as though material had been utilized from them in the growth of the plant.

#### FUTURE DEVELOPMENT OF PLANTS

All plants failed to maintain an uninterrupted shoot growth beyond 50 to 70 mm., as contrasted with plants which develop from after-ripened peach seeds and reach a height of 300 to 400 mm. during the growing season. The cessation of terminal development and failure of the axis to elongate resulted in all cases in plants with at least some degree of dwarfing. Plants from embryos excised at the earliest stages were most dwarfed, and those from later stages least dwarfed. These last appeared similar to those described by FLEMION (5) from non-after-ripened embryos of mature peach seed.

When placed in the greenhouse in soil, the plants remained in this stage of arrested development, the stem became woody and some leaves abscised as when plants enter a period of dormancy. They were then placed in a nursery cellar which provided subdued light and a temperature of about 45° F. In 30 days the plants were returned to the greenhouse. They immediately resumed rapid growth (fig. 14D), free from any abnormalities, such as characterized the earlier stages of development. Whether a shorter period of time would have accomplished the same results or what other factors might have brought about the same results is not known, since no other treatments were used. Close examination of the new shoot growth showed that it arose not from the terminal bud but from a lateral bud in the axil of one of the leaves near the tip of the shoot, as already described by DAVIDSON (2).

Subsequent behavior of the seedlings was similar to that of seedlings from after-ripened seeds. Planted into the orchard they grew into trees, some of which have borne fruit at 4 years of age. General fruit and tree characters were "normal," the only differences being the variations in individuals to be expected among seedling peaches.

The youngest embryos which were successfully cultured and grown into orchard plants were those excised 73 days after full bloom, and 56 days before the fruit was ripe. Considerable difficulty was experienced with damping-off fungi. Embryos 80 days old from full bloom were raised to orchard plants much more easily but still were difficult to handle because of the fungi. From 94 days to 129 days after full bloom the stand of seedlings was nearly perfect and little difficulty was experienced in growing the plants to orchard trees, some of which have fruited.

#### **Results with other varieties of peaches during different seasons and from different sources**

The embryos of the other four varieties of peaches received from Georgia behaved, in general, similarly in culture to the embryos of Elberta. A few points of difference are worth noting. First there was a spread of several days in blooming between the different varieties, in which Greensboro bloomed first and Elberta last. Since the rates of development of the fruit, seed, and embryo are nearly identical for all varieties, it follows that development of Greensboro was several days in advance of Elberta on the same calendar date. For example, 55 days after full bloom, embryos of Greensboro were about one-third maximum size; those of Carman, one-quarter; those of Hiley, one-quarter; those of Belle, one-quarter; and those of Elberta, one-sixth.

The response of the embryo in culture reflected the more advanced development of some varieties over others on the same date. For example, 61 days after full bloom the separation of the cotyledons and the development of chlorophyll was greatest in Greensboro, next greatest in Carman, and least in Elberta. Similarly, 73 days after full bloom, all the embryos of Greensboro and Carman had developed chlorophyll after 10 days in culture, as compared with one-third of

the embryos of Hiley, and one-fifth of those of Belle and Elberta. This relationship between varieties continued throughout the season.

Embryos of the early-ripening varieties aborted before they completely filled the integuments. Such embryos, although cultured at intervals for several weeks thereafter, developed only to the degree characteristic of embryos at the time of aborting. That is, embryos of Greensboro which had aborted 61 days after full bloom, and which were cultured 19 days later, developed in culture only to the stage of spreading the cotyledons and forming chlorophyll, as is characteristic of 61-day embryos. Embryos of Elberta, being non-abortive, behaved in culture on this date similar to 80-day embryos, roots frequently forming 5 to 20 mm. in length and the central axis of the epicotyl growing to 7 to 15 mm. in length.

The fact that the samples of peaches received from Georgia were several weeks further developed than those from Geneva, New York, and those from Geneva several days further than those from Youngstown, New York, gave an interesting comparison of embryos of the same variety cultured on the same day but in different stages of development. In all cases the embryos developed characteristic of the stage of development at which they were excised, so that strong shoot and root development appeared from embryos received from Georgia, whereas embryos from peaches from Geneva cultured on the same date developed only short shoot growth, and those from Youngstown developed only to the white rosette stage.

In addition to the varieties mentioned, embryos of the following thirty-one varieties, cultured during the seasons of 1932 to 1936, and secured from both Youngstown and Geneva, New York, representing a wide range of climatic and cultural conditions, seasonal development, and season of fruit ripening, gave substantially the same results: Alexander, Alexander Crosby, Arp, Belle, Blood Leaf, Canada, Carman, Champion, Chili, Crosby, Delicious, Eagle Beak, Early Crawford, Elberta, Foster, Golden Jubilee, Greensboro, Krummel, Lola, Mikado, Morellon, Mountain Rose, Rochester, Rosebud, St. John, Triumph, Troth, Valiant, Veteran, Waddell, and Ward Late.

### Results with embryos of cherry, plum, apricot apple, and pear

The results in culturing excised embryos of the sweet cherry, sour cherry, plum, apricot, apple, and pear indicate the same general trend for these classes of fruits as for the peach, the species of *Prunus* following a little more closely than the apple and pear.

For a given species, the response of the embryos in culture is in accordance with the characteristic curve of growth of the embryo for that species. That is, the embryo of the sweet cherry begins its period of rapid development 17 days after full bloom; the sour cherry 21 days; the peach 49 days; the apricot 42 days; the apple 35-40 days; and the pear 40 days.

Accordingly, embryos of the sweet cherry responded in culture at an earlier calendar date than did embryos of the peach. When excised embryos of the peach had later reached the same stage of development as that of the cherry previously cultured, the growth in culture was similar.

Within a given species, embryos of the various varieties used behaved similarly, with the minor differences to be mentioned in the following paragraphs.

#### APRICOT

Embryos of the variety of apricot used, Alexander, behaved similarly to those of the peach. They developed vigorously in culture and developed into strong plants.

#### SWEET AND SOUR CHERRY

Embryos of the cherry responded more slowly in culture than embryos of the peach and the resulting plants were more delicate, less vigorous, and less easily handled. Using the embryo of the Mazzard cherry as the type, embryos excised prior to 27 days after full bloom, which had by that time reached a length of 1.48 mm., failed to develop in culture (fig. 15A). Beginning with embryos 30 days of age from full bloom, when the embryos had reached a length of 2.6 mm. compared with a maximum length of 7.2 mm. at maturity, the first indications of development were secured. The cotyledons of such embryos spread apart but developed no further. Embryos excised 32 days after full bloom, 3.1 mm. in length, developed

cotyledons which were thick and green, and two small white recurved leaves (fig. 15*B*).

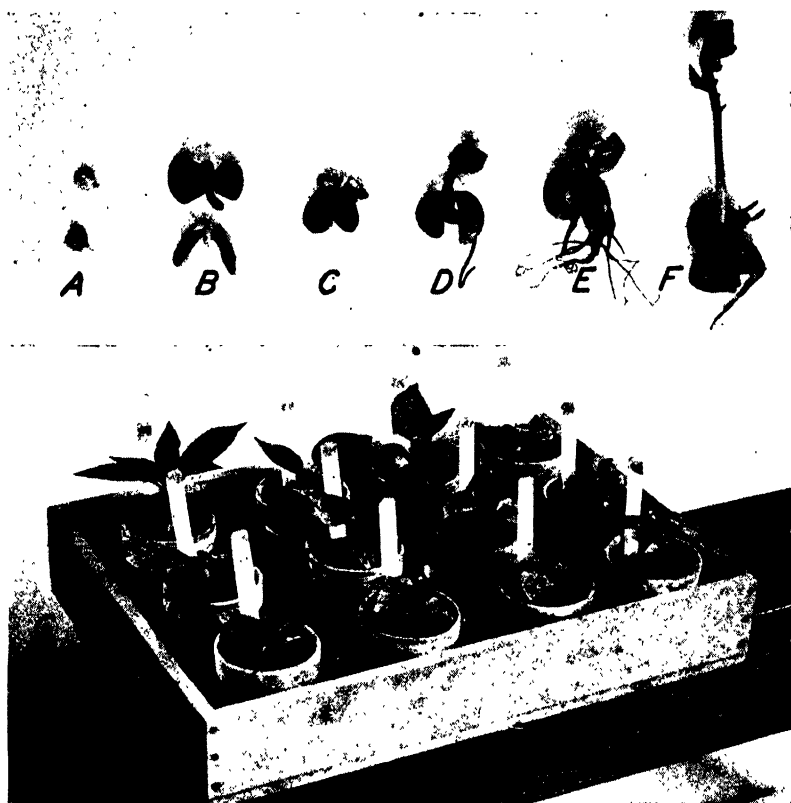


FIG. 15.—Growth in culture of sweet cherry embryos excised from fruit at various stages in development, time in days after full bloom: *A*, 27 days, no development in culture; *B*, 32 days, cotyledons spreading, thickened, and green, with two small white recurved leaves; *C*, 34 days, epicotyl terminated in rosette of small, white, stipule-like leaves; *D*, 36 days, hypocotyl thickened and elongated, roots developing, epicotyledonary axis elongating 2 to 4 mm., terminated in rosette of small, white, stipule-like leaves; *E*, 40 days, roots and small green leaves developing; *F*, 45 days, epicotyledonary axis elongated, leaves formed; *G*, 54 days, vigorous plant developments but with broad, crinkled, anomalous leaves and dwarfish habit of growth.

Embryos excised 34 days after full bloom, which were then 4.42 mm. in length, developed chlorophyll in the cotyledons in culture, the hypocotyl lengthened to 2–4 mm., and the epicotyl developed

as a rosette of small, stipule-like leaves in which no chlorophyll formed (fig. 15C). Thirty-six days after full bloom the embryos had reached a length of 5.8 mm. In culture they produced roots 3 to 5 mm. in length, developed chlorophyll in the cotyledons, and produced an epicotyledonary axis 2 to 4 mm. in length, surmounted by a rosette of stipule-like leaves in which chlorophyll was now developed (fig. 15D). Forty days after full bloom embryos had reached a length of 6.9 mm., which in culture developed roots and small green leaves (fig. 15E). Embryos excised 45 days after full bloom had reached maximum size, and developed in culture into plants with good root development, elongated epicotyledonary axis, and green leaves (fig. 15F).

Embryos excised 54 days after full bloom developed into vigorous plants, but which had broad, crinkled, anomalous leaves quite unlike a typical cherry leaf. Further, the habit of growth was dwarfish. As with the peach, such plants, placed at a temperature of 45° F. for 30 days in subdued light and then returned to the greenhouse, began a new shoot growth which no longer showed any anomalous growth forms or any dwarfish characters (fig. 15G).

Other varieties of sweet cherry used were Early Purple Guigne, Black Tartarian, Lyons, Seneca, Rockport, Kirtland, Burbank, Oswego, Yellow Spanish, Windsor, and Schmidt.

Sour cherries used were Early Richmond, English Morello, and Brusseler Braune. They behaved similarly to the sweet cherry. The embryo of the sour cherry, however, is less fully developed than that of the sweet cherry on the same date. Accordingly embryos of the sweet cherry in culture were developing into normal plants on the same day that embryos excised from the sour cherry were only beginning to develop chlorophyll in the cotyledons.

As with the peach, the earliest ripening varieties produced abortive embryos. The earliest, Early Purple Guigne, aborted so early that the embryos seldom reached the stage at which they developed in culture. Only occasionally was an embryo of the Early Purple Guigne found sufficiently advanced to develop.

An interesting observation in this connection was made with embryos of varieties ripening in succession and all cultured on the



same date, namely, Seneca, Burbank, Knight, and Black Tartarian. The embryos of the earliest ripening variety, Seneca, having aborted at a very early stage, developed only to the degree characteristic of that stage. Embryos of the next ripening variety, Burbank, having aborted at a slightly later stage, developed more fully in culture. Embryos of Knight, which ripens still later, developed still further in culture; and embryos of Black Tartarian, the latest ripening of the group, developed the furthest.

### PLUM

Embryos of the varieties of the European plum Oullins, Italian Prune, and Middleburg responded in culture as well as, and similarly to, embryos of the peach. Of these three varieties, Oullins ripens early, Italian Prune in mid-season, and Middleburg late.

Unlike the varieties of peach, which all came into full bloom on the same date, thus giving a similar embryo development for all varieties on a given calendar date, Middleburg blossoms earliest, Oullins next, and Italian Prune last. On the same calendar date, therefore, embryos of Middleburg developed most fully in culture, Oullins next, and Italian Prune least, thus corresponding to the succession in bloom. But, computed on the basis of days following full bloom, embryos of all three developed in culture as characteristic of the interval following full bloom at which they were excised.

No development in culture was observed from embryos excised prior to 61 days from full bloom. Excised at the following intervals after full bloom, the characteristic development of embryos was as follows: 69 days, chlorophyll developed in the cotyledons; 72 days, hypocotyl elongated 2 to 4 mm. and epicotyledonary leaves recurved but remaining white; 78 days, roots developed and a rosette of small whitish stipule-like leaves formed; 83 days, epicotyledonary axis elongated to 2-5 mm. and the rosette of stipule-like leaves becoming green; 86 days, stem elongated to 15 mm.; 90 days, both root and shoot growth vigorous but elongated stem terminating in a rosette of green leaves; 98 days, vigorous plant development.

As in the peach, all plants were dwarfish, and when placed at a temperature of 45° F. in subdued light for 30 days, they began new and normal growth.

Embryos of the American varieties Tecumseh and DeSoto developed in similar stages, but the plants grew more feebly. Tecumseh came into full bloom a few days earlier than DeSoto, so that excised embryos of the former were always a few days in advance of those of the latter and behaved in culture as characteristic of that stage.

#### APPLE AND PEAR

Embryos of the apple and pear failed to develop as vigorously in culture as did those of the species of *Prunus*, particularly as those of the peach and apricot. The type of plant into which they developed was characteristic of the age from full bloom at which they were excised, and was similar to those already described for the peach. Shoot development was slender and weak, leaves were small, and roots were slender and lateral roots seldom developed.

The stage of development which embryos of the pear and apple reached on the tree at a given calendar date were sometimes variable, adding to the difficulty of an accurate interpretation of results. Unlike the peach, different varieties of which reach full bloom on nearly the same day, different varieties of apple and pear reach full bloom at successive intervals over a period of a week to 10 days, or sometimes longer. Furthermore, apple flowers are borne in a corymb and those of the pear in a cyme, so that there is a difference in time of bloom of individual flowers in the same cluster. Also, the flowers may be borne terminally, laterally, and on spurs—all on the same tree—and all varying slightly in the time at which they reach full bloom. WHITEHOUSE (30) has shown that fruits which are of the same size in early season develop at the same rate. No doubt tagging of blossoms which bloom on the same day, or hand pollination, would result in more uniform material for culture.

Nevertheless the results were consistent with embryos of the same age. As in the case of the plum, embryos of varieties which reached full bloom earliest were furthest advanced on a given calendar date and responded in culture accordingly. McIntosh, for example, bloomed 6 days earlier than Rome Beauty. Embryos of McIntosh excised on the same day as embryos of Rome Beauty developed into plants more advanced in type. Yet embryos of Rome Beauty excised 6 days later developed into plants of similar type.

APPLE.—Apple varieties used were Red Astrachan, Early Harvest, Maiden Blush, R. I. Greening, McIntosh, and Rome, representing a wide range in season of ripening. Early Harvest embryos were consistently further developed the same number of days after bloom than embryos of the other varieties used. Since the cultivated apple is a combination of several species, this fact may account for the variation; or it may be that embryo development in varieties of apple is not so uniform as in the case of other fruits studied.

The other varieties of apple developed similarly. No development in culture was observed from embryos excised prior to 39 days from full bloom. Excised at the following intervals after full bloom, the characteristic development was as follows: 45 days, spreading of cotyledons and slight development of chlorophyll; 59 days, cotyledons dark green; 62 days, hypocotyl 2 to 4 mm. long, epicotyl developing into rosette of small, white leaves (fig. 16); 69 days, hypocotyl 6 mm. in length, epicotyl developing as rosette of small white and green leaves (fig. 16); 75 days, roots 10 mm. in length; 86 days, cotyledonary axis elongated to 4–6 mm., surmounted by small rosette of small white and green leaves, roots 20 to 25 mm. in length (fig. 16); 92 days, plants developed sufficiently to be grown in the greenhouse; 99 days, roots 25 mm. in length, epicotyledonary axis 8 mm. in length, surmounted by small rosette of green stipule-like leaves; 105 days, roots 25 to 30 mm. in length, stem 18 mm. with one or two slender leaves 6 mm. long; 120 days, normal but weak root, shoot, and leaf development; 134 days, stem 45 mm. in length, normal but weak shoot and root development.

It should be observed that no plants developed into normal seedling growth unless chlorophyll formed in the epicotyl. Chlorophyll development in the cotyledons and the formation of white leaves were not enough to carry the plants further.

PEAR.—Pear varieties used were Tyson, Seckel, Bartlett, and Kieffer, representing a wide range in season of fruit ripening. The first three are pure *Pyrus communis* L., the last is a hybrid between *P. communis* and *P. serotina*.

No development was observed in culture from pear embryos excised prior to 46 days from full bloom. Excised at the following

intervals after full bloom, the characteristic development was as follows: 66 days, cotyledons green; 69 days, cotyledons green, epicotyledonary axis 1 to 2 mm. in length, terminated in small rosette

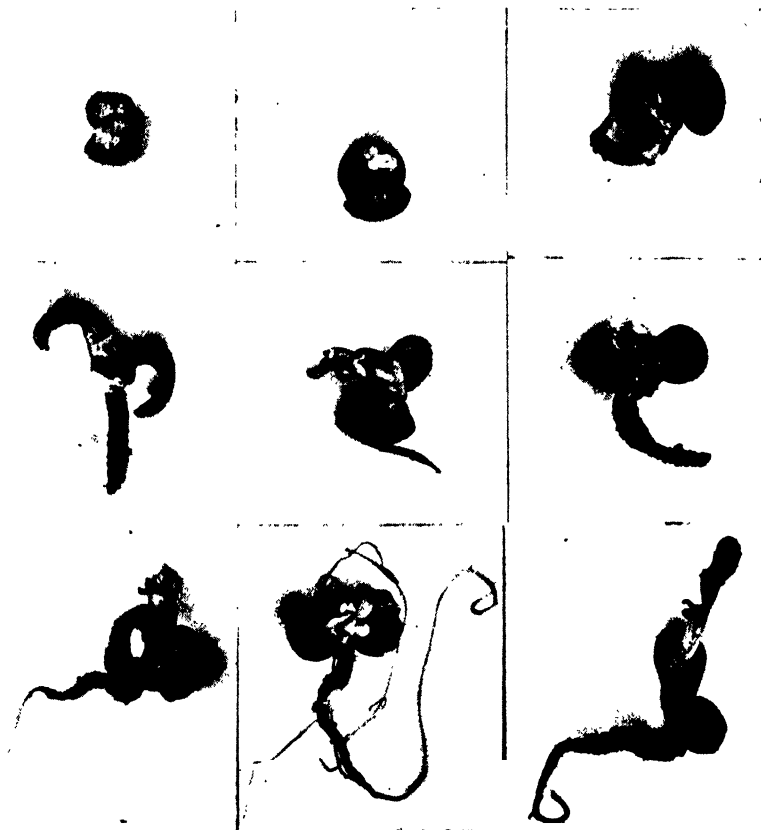


FIG. 16.—Growth in culture of apple embryos excised from fruit at various stages in development: Top row, 62 days after full bloom; cotyledons green, hypocotyl 2 to 4 mm. in length, epicotyl terminating in rosette of small, white leaves. Middle row, 69 days after full bloom; hypocotyl 6 mm. in length; epicotyl developing as rosette of small white and green leaves. Bottom row, 86 days after full bloom; epicotyledonary axis 4 to 6 mm. in length, surmounted by small rosette of small white and green leaves; roots 20 to 25 mm. in length.

of small white and green leaves (fig. 17*A, B, C*); 75 days, stem slender and 6 to 8 mm. in length, leaves slender and stipule-like, roots 22 mm. in length, plants developed sufficiently to be grown in the green-

house (fig. 17*D, E, F, G*); 81 days, stem slender and 18 mm. in length, leaves slender, roots 40 mm. in length; 97 days, stem weak and slender, 25 mm. in length, leaves normal but small and thin, roots 55 mm. in length (fig. 17*H, I*).

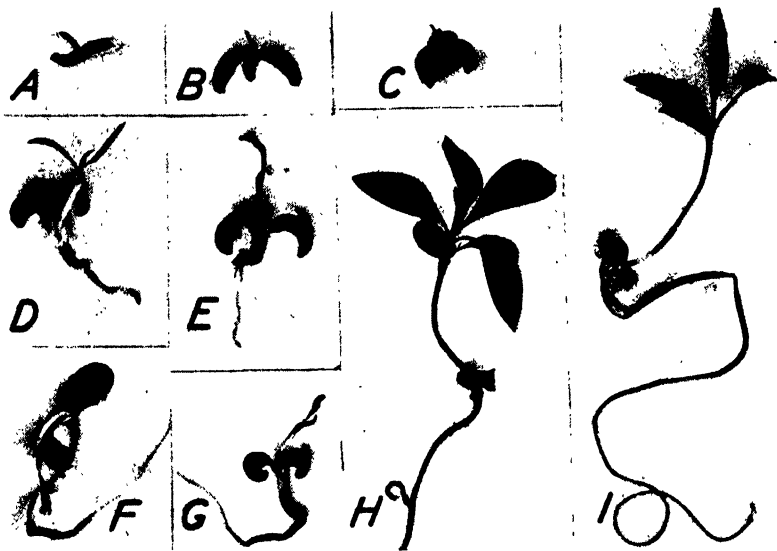


FIG. 17.—Growth in culture of pear embryos excised from fruit at various stages in development: *A, B, C*, 69 days after full bloom; cotyledons green, epicotyledonary axis terminated in small rosette of small white and green leaves; *D, E, F, G*, 75 days after full bloom; stems slender, leaves slender, roots 40 mm. in length. *H, I*, 97 days after full bloom; stem weak and slender, leaves normal but small and thin, roots 55 mm. in length.

### Results without use of disinfecting agent

While the use of a disinfecting agent lends itself well to large scale methods of embryo culture, the question is immediately raised as to what effect such an agent may have upon the embryos. Of several materials used, namely, zonite, bichloride of mercury, and calcium hypochlorite, the last has proved very close to ideal. It is easily prepared according to the formula of WILSON (31) and as used by KNUDSON (10) for cultures of orchid embryos, giving almost exactly 20,000 p.p.m. of chlorine (2 per cent). It is inexpensive and easily handled, and immersion of embryos for 5 minutes has given almost perfect freedom from contamination.

On the other hand, it has been fairly simple to dissect embryos under aseptic conditions and to place them in culture without the use of a disinfecting agent. With the use of a transfer room, contamination has been reduced to 1 or 2 per cent. DAVIDSON (2) has found a transfer case helpful, and good results may be secured in the open laboratory provided proper precautions are taken. Both

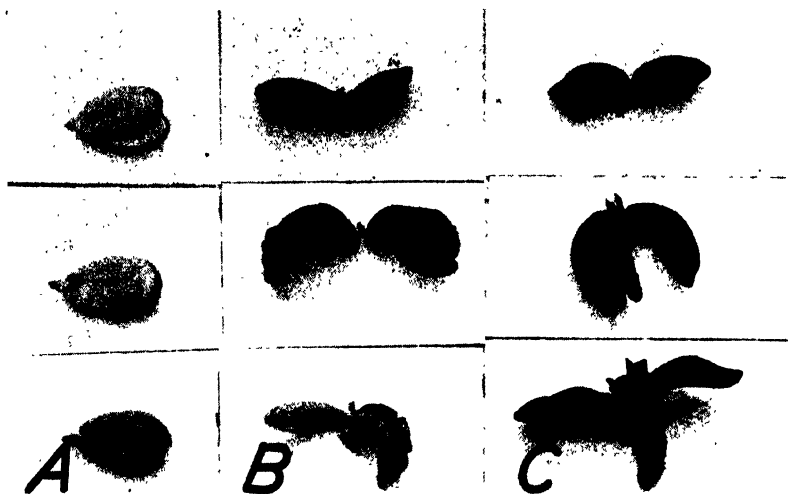


FIG. 18.—Effect of glucose upon apple embryos excised at early stages, showing that glucose is essential to chlorophyll formation and that higher concentrations are best for embryo development at early stages: *A*, no glucose in medium, no development; *B*, 0.5 per cent glucose, partial development; *C*, 2 per cent glucose, most development. Cf. fig. 19.

aseptic and disinfectant methods have been found practical and useful, depending upon the material used and the objectives desired.

The behavior of embryos placed in culture without treatment with a disinfecting agent differed in only minor points from the general behavior of embryos treated with calcium hypochlorite, as described in preceding paragraphs. Embryos excised at very early stages of development, 48 days after full bloom or earlier, failed to develop following treatment with calcium hypochlorite solution, although when embryos of this age were dissected in broth of the medium and then placed in culture, the cotyledons had spread

apart within 8 days and the embryos increased from a length of 0.9 mm. to 3.8 in 21 days. None developed into greenhouse plants.

With older embryos 51 to 94 days from full bloom, treatment with calcium hypochlorite delayed the formation of chlorophyll in the cotyledons 3 to 6 days, particularly on outer surfaces of the cotyledons, which were most exposed to the action of the material. In some instances, when embryo treatment was extended to 10 or 20

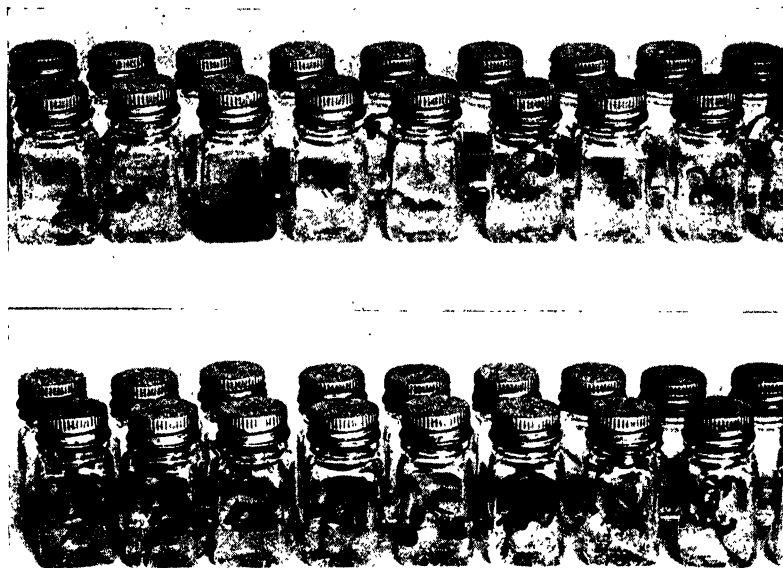


FIG. 19.—Effect of glucose upon cherry embryos excised at late stages, showing that glucose is inhibiting to the development of late stages: above, 2 per cent glucose in medium, poor growth; below, no glucose in medium, good growth. Cf. fig. 18.

minutes, chlorophyll failed entirely to develop on the dorsal surfaces of the cotyledons, although it later formed on the ventral surfaces after the cotyledons had spread apart.

With still older mature embryos, 100 days or more from full bloom, there seemed to be no effect upon development from treatment with calcium hypochlorite solution for 5 minutes.

In general calcium hypochlorite solution has been injurious to very young embryos, retarding to older embryos, and non-injurious to still older embryos.

### **Relation of medium to age of embryo when excised**

The composition of the medium has affected variously the development of embryos excised at different stages. In the results that have been listed as the type in the present paper, the standard medium given in earlier paragraphs has been used. Varying several factors in the medium within the limits described, such as the pH and the composition and concentration of mineral nutrients, has had no appreciable effect upon embryo development. But the concentration of glucose has had a decided effect. In the case of early stages of embryo development, the presence of glucose in the medium is essential to chlorophyll development in the cotyledons. Further, although 0.5 per cent concentration of glucose favors development of both chlorophyll and the embryo, 2 per cent produces a still greater response. By the use of this higher concentration of glucose for embryos excised in early stages of development, still earlier stages can be successfully cultured.

By contrast, in late stages of embryo development, as when embryos have reached maturity, the presence of 2 per cent glucose in the medium is inhibiting. By the use of lower concentrations or by the elimination of glucose, still later stages may be successfully cultured.

By the use of 0.5 per cent glucose for the results presented in this paper, the range has been extended from early to late and has tended to give a more complete story than would otherwise have been the case.

Nevertheless the fact remains that by varying the concentration of glucose and by its absence from the medium, development of embryos has been altered. It is not too much to expect that with a more complete understanding of the chemical make-up of embryos at various stages in their development, and of the nutrients required, and with improved technique and methods of manipulation, still further progress may be made in altering or even eliminating some of the characteristic stages of development of embryos excised at various periods after full bloom and bringing them more nearly to what might be called normal plant development.



### Discussion

The culturing in artificial media of immature embryos excised from growing plants has practical application to plant breeding and genetics, as the perpetuation of individuals in a given population which might otherwise fail to survive (12, 18). Further, it provides a method for studying the new sporophyte at earlier stages in development than that provided by the mature seed, and serves to focus attention upon the first expression of the new individual in the embryo rather than upon the plant developed from a mature embryo (22).

Throughout these studies, the stage of development of the embryo when excised from the fruit has overshadowed the other factors considered, but size alone is not a satisfactory criterion of whether or not an embryo can be grown successfully in culture. That chemical composition must be considered as well as size is shown by chemical analyses of developing peach embryos by TUKEY and LEE (24). They have shown that the peach embryo reaches nearly maximum size before any appreciable accumulation of fat (ether extract), nitrogen, and sugars begins. With embryos 17.5 mm. in length the content of fat was 0.40 per cent, of nitrogen 0.68 per cent, and of sucrose 1.06 per cent, whereas at maturity the embryos were only 1.5 mm. greater in length; but the content of fat was 30.67 per cent, of nitrogen 2.60 per cent, and of sucrose 2.32 per cent.

It would seem that a better comparison would be the age of the embryo on the basis of time, the differentiation of the embryo on the basis of morphological characters, or the development of the embryo on the basis of chemical composition.

That some of the youngest embryos did not respond to culture methods is not surprising, but that each age was expressed in a characteristic growth pattern is of particular interest. A similarity is at once recognized between the growth patterns presented in this paper and well known "juvenile" and "adult" forms in other plants. In discussing the differences in the formation of organs at different developmental stages, GOEBEL (7) emphasized the fact that all living things are in a condition of constant change, from earliest to latest stage in development. He cited differences in both function and in conformation of plant parts running through all plant phyla.

These facts emphasize that it is not the genetic make-up of an individual alone which is responsible for the expression of a plant in the formation and function of its various parts. As GOLDSCHMIDT (8) has explained, there are “. . . two general notions in regard to the causal understanding of individual development. . . . One is the notion . . . that the action of the genes in controlling development is to be understood as working through the control of reactions of definite velocities, properly in tune with each other and thus guaranteeing the same event always to occur at the same time and at the same place. . . . The second notion . . . says that two types of differentiation are closely interwoven in the process of development, namely, independent and dependent differentiation. Independent differentiation means that a once started process of differentiation takes place within an organ or part of the embryo, even if completely isolated from the rest; dependent differentiation, however, requires the presence and influence of other parts of the embryo for orderly differentiation.”

Of course it may be that the growth patterns described in this paper are merely the response of the plant to an unnatural and unfavorable environment. In a sense they may be considered mal-adjusted plants. The younger the embryo, the greater the difficulty in culturing and the greater the abnormal behavior. The older the embryo at the time it is excised from the mother plant, the more fully differentiated it has become and the less easily it is upset or thrown out of balance by an unnatural environment. The facts that a liquid medium is favorable at one stage of development and not at another, and that glucose is favorable at one stage and inhibiting at another, suggest that by providing a more suitable environment, by better technique, and by a better understanding of nutritional requirements, some of these growth patterns may be altered to more nearly the normal for the plant. On the other hand, the fact that even mature seeds must be after-ripened before they develop normally, suggests that there is an internal complex as well as an external environment which must be considered.

The failure of embryos to follow the pattern of embryonic development outside the environment of the mother plant raises the question as to what is the nature of the environment which brings

about "normal" embryo development on the plant. The shape of the embryo, it has been shown by HANNIG and others, as well as by these studies, is altered by its surrounding tissue. Does this mean that it is the shape of the campylotropous seed which physically causes a bean embryo to develop its curved shape? Or does it mean that some nutritional factors control the whole?

Finally, from the standpoint of developing mature plants from immature embryos, the facts reported in this paper point toward greatest success by maintaining an embryo in its natural environment or as nearly a natural environment as possible, so that it may follow the normal course of embryonic development before being subjected to a less favorable or less natural one.

### Summary

1. Methods and results are given in culturing embryos of twelve varieties of sweet cherry (*Prunus avium*), five of sour cherry (*P. cerasus*), three of European plum (*P. domestica*), two of American plum (*P. americana*), thirty-two of peach (*P. persica*), one of apricot (*P. armeniaca*), five of apple (*Malus domestica*), and four of pear (*P. communis* and *P. communis*  $\times$  *P. serotina*), during five growing seasons, 1932 to 1936 inclusive. More than 20,000 individual cultures have been made. Material has been cultured from Georgia and from three locations in New York State.

2. Embryos in culture do not pass through the embryonic stages characteristic of embryos on the mother plant. Instead they enter at once into an independent development characteristic of the age of the embryo when excised.

3. The growth pattern for peach embryos treated with a disinfectant and grown on 0.6 per cent agar media with 0.5 per cent glucose and salt mixture T may be summarized as follows: (A) no development earlier than 51 days of age after full bloom; (B) 51 days, spreading and greening of the cotyledons and small white epicotyledonary leaves; (C) 73 days, cotyledons green, hypocotyl 2 to 4 mm. and roots 10 to 15 mm. in length, central axis of epicotyl 1 to 2 mm. in length terminated by rosette of six to ten small white stipule-like appendages; 80 days, roots 10 to 20 mm. in length, central axis of epicotyl 7 to 15 mm. in length surmounted by rosette of

eight to twelve anomalous, white, stipule-like appendages; (D) 87 days, vigorous root development, central axis of epicotyl 20 to 22 mm. in length terminated by rosette of green stipule-like appendages; (E) 94 days, vigorous root development, central axis of epicotyl 25 to 30 mm. in length terminated by rosette of small, green stipule-like appendages, occasional peachlike but malformed leaves; (F) 105 days, vigorous root formation, stem 40 to 45 mm. in length terminated by rosette of small, green stipule-like leaves, with peachlike leaves along the stem; (G) 108 days, vigorous root and shoot development but leaves often broad and crinkled and plants dwarfish; (H) 118 days, vigorous root and shoot growth but dwarfish plants.

4. After 30 days in subdued light at 45° F., dwarfish plants began normal development and showed no further abnormal behavior.

5. Embryos of sour cherry, sweet cherry, apricot, plum, apple, and pear behaved similarly with minor differences.

6. Aseptic methods resulted in earlier response than when a disinfecting agent was used, but growth patterns were similar. Very young embryos were injured by a disinfectant.

7. Growth patterns were modified by altering the medium, especially glucose, in which at early stages of development glucose was beneficial and at later stages inhibiting.

8. The data are discussed with reference to physiological changes in the embryo itself, juvenile and adult forms of plants, and general problems of morphogenesis.

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## CURRENT LITERATURE

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*Marine Algae of the Northeastern Coast of North America.* By WILLIAM RANDOLPH TAYLOR. Illustrated by CHIN-CHIH JAO. University of Michigan Press, 1937. Pp. 427. Illustrated.

Botanists and others interested in the marine algae of the northeastern coast of this continent have long been at a disadvantage, because there has been no comprehensive treatment of them since FARLOW published his *Marine Algae of New England*, nearly fifty years ago. This need has been met in a very adequate manner by Professor TAYLOR's treatise covering the marine algae ranging from Virginia to the eastern American Arctic, including Hudson Bay. In large part the book presents the results of the author's study of living algae along the eastern shores of this country, especially in the region centering around Woods Hole, Massachusetts; his reexamination of the major phycological collections in this country, especially those of FARLOW and of COLLINS; and his study in European phycological herbaria, notably those of HARVEY and the younger AGARDH.

The book begins with an introductory account of the geographical distribution of marine algae within the area, and with a very useful sketch of the methods of collecting and preserving marine algae. The major portion is devoted to a systematic account of the algae. This is arranged in the conventional pattern, and with descriptions of orders, families, genera, and species, the last with habitat and geographical range indicated in some detail. There are also critical notes on many of the species. Keys are given for each order, family, and genus.

The descriptions of the various families and genera are noteworthy in that they incorporate modern studies on the life cycles and on the anatomy of reproductive organs. In the opinion of the reviewer the value of these descriptions would have been enhanced by citation of papers on anatomy and on life history studies, immediately following the descriptions of families and genera. As placed in the book these references are all listed according to species only, and intermingled with taxonomic references.

Most of the descriptions of the various species are original and give the author's concept of the limits of the species. In this he leans toward conservatism. He has also taken a conservative attitude on the distinctiveness and similarity of species among genera found on both American and European shores of the Atlantic.

A general key has been purposely omitted because the author thinks that, "In practice, the gross aspects of the common genera of marine algae are so readily learned that after a few days at the seashore the observer should be able to resort directly to the keys under the genera." In the opinion of the reviewer,

artificial keys, such as KYLIN has given for the Rhodophyceae of Puget Sound, are decidedly helpful to the beginning phycologist, to whom every alga is a systematic problem because there are no evident features showing its order or family. Dr. JAO's admirable illustrations compensate in the main for this lack of a key. These drawings include habit sketches of most of the genera and important details of anatomy of many of them, and are the best that have been published in the last fifty years.

American botanists are to be congratulated that the "standard book" on the marine algae of their Atlantic Coast has been done in so excellent a manner.—G. M. SMITH.

*The Genus Bidens*. Parts I and II. By EARL EDWARD SHERFF. Field Museum of Natural History, Bot. Ser. 16, 1937. Pp. 709. Illustrated.

The modest title of this publication scarcely indicates the scope of the work; it embodies the results of a comprehensive and critical study of the genus *Bidens*, a relatively large group of plants and a conspicuous and wide-spread element of the world's flora. As the author states, "The genus *Bidens* was so closely intertwined in botanical literature with *Coreopsis*, *Cosmos*, *Coreocarpus*, *Megalodonta*, *Dahlia*, *Isostigma*, *Heterosperma*, *Thelesperma*, and certain other genera of Compositae, that it became necessary in many cases to make a truly monographic study of these allied genera before attempting to progress further in the treatment of *Bidens* itself."

The preface contains a long list of institutions which have furnished material for study and of individuals who have co-operated in one way or another with the author in the course of his work. This fact, combined with the widely circulated preliminary publications on the group, gives ample evidence of the general interest aroused as well as an indication of the need for a comprehensive, uniform, and single taxonomic treatment of the group of plants concerned. A historical survey of the genus is followed by a section including morphology, histology, cytology, ecology, etc.

The main thesis is concerned with taxonomy. Fourteen sections of *Bidens* are enumerated and defined. Two hundred and thirty-five species, about 120 varieties, several forms, and a few hybrids are recognized as valid taxonomic entities. About 650 scientific names in various combinations are reduced to synonymy. The valid species are grouped for convenience into five geographical categories, as follows: Plants native to islands of Central Pacific Ocean; Plants growing in North and Central America and in the West Indies; Plants of South America; Plants of the Eastern Hemisphere, excluding Africa; Plants of Africa.

Under each of these geographical categories there is a dichotomous key, based on the more evident morphological characters leading to the species. Full and lucid descriptions are given of the species, varieties, and forms. The type specimen of each taxonomic unit, as well as the herbarium in which it is deposited, is indicated. Collections are copiously cited. Pertinent notes and comments are appended. The numerous illustrations, which have been faithfully



and carefully delineated by the author from types or authentic specimens, greatly aid in visualizing the characters recorded in descriptions.

A list of about 100 names to be excluded from *Bidens*, descriptions of two new species, a few names of doubtful status, and an index of collectors cited conclude the monograph.

This work is a distinct contribution to the literature of botanical science, and will constitute an authoritative reference work for many years to come.—J. M. GREENMAN.

*A Textbook of Plant Virus Diseases.* By KENNETH M. SMITH. Philadelphia: Blakiston, 1937. Pp. 615. Illustrated.

Further evidence that the field of phytovirology is coming of age as a science is the fact that plant viruses have been worked sufficiently to permit their classification and the writing of an excellent textbook on plant virus diseases, with classification of the viruses as its principle of organization.

Chapters 1-7 deal with viruses, beginning with Delphinium virus 1 and ending with Oryza virus 2. So far as possible (attained most completely for Nicotiana virus 1, and other Solanum viruses) the discussion of each virus deals with its physical and chemical properties, methods of transmission, differential hosts, diseases caused by it (arranged according to host families) including histopathology, and strains for each of which the preceding presentation is carried out as far as possible.

Chapter 8 deals with insects, etc., concerned in transmission of plant viruses and chapter 9 with suspected virus diseases requiring further investigation. An appendix in tabular form gives the names (scientific and common) of the host plants affected, symptoms induced, and the name of the disease incited by each virus. There are addenda, a general index, an index of viruses, and one of authors. Literature is cited at the close of each chapter. The illustrations are abundant and high grade.

This excellent book will be welcomed, not only by teacher and pupil, but also by the large number of researchers who are "discovering" viruses with appalling frequency, and the practical men who are attempting to stem the tide of virus creation.—G. K. K. LINK.

*Introduction to Plant Pathology.* By F. D. HEALD. New York: McGraw-Hill, 1937. Pp. 578. Illustrated.

This book is more simply written and covers less material than its excellent predecessor, HEALD's "Manual of plant diseases." As a result it is more available by elementary students but also less desirable for advanced investigators. It is characterized by the meticulous workmanship, accuracy, and clarity of the larger book, and will be welcomed by teachers of plant pathology and those seeking an introduction to the subject.

It is unfortunate that the author did not retain the order of presentation used in the Manual. In that work HEALD followed the pedagogically and theo-

retically sound principle of presenting first the non-parasitically induced diseases, and then the infectious diseases. This treatment might loosen the hold of the germ theory of disease as the cardinal principle in the experimental procedure of American and English plant pathologists. By reversing the order, especially in an introduction to the subject, the author elevates infection, which is only a special case of the class injury, to the position of central or leading concept, and degrades phytopathology to the position of only one phase of parasitology. A further lapse into, or a concession to, the characteristic conception and practice of phytopathology as a mycological discipline is evident in the fact that discussion of fungus induced diseases, with which the presentation of diseases is begun, is preceded by a chapter on the relation of fungi and bacteria to human affairs, in which considerable space is devoted not only to harmful relations in processed food stuffs, cheese, butter, leather, fabrics, and in animals, but also to useful relations of fungi.

The book, like its predecessor, designates plant pathologists "plant doctors," "medicine men of agriculture." Are these happy choices of terms? Those practicing the art of plant or crop protection may be designated plant doctors, if one must use such a label, but what about those engaged in investigations which are not oriented toward control, but toward advance of our knowledge about the basic biologic problems of injury and reaction to injury? By adopting this narrow conception of the rôle of the phytopathologist and implanting it in the minds of beginners who will be the leaders of the next generation, is not American phytopathology in danger of selling its birthright of a basic biologic science, rich with socially significant ideas, for the mess of pottage of a narrowly delineated art?—G. K. K. LINK.

*Selected Topics in Colloid Chemistry.* By ROSS AIKEN GORTNER. Ithaca, New York: Cornell University Press, 1937. Pp. xiii+169. Illustrated. \$2.50.

During the first semester of the year 1935-36, the author filled the George Baker Fisher Non-Resident Lectureship in Chemistry at Cornell University. These lectures are now presented to the public in the form of a very attractive book. The first lecture, which serves as an introduction, bears the title Scientific Genealogy. It gives an intimate personal glimpse of the author's contacts with his teachers, an example of the spiritual genealogy of all scientists.

The chemical lectures are eight in number, with titles as follows: The beginnings of science; what is colloid chemistry; some basic concepts; some fundamental properties of colloidal systems; electrokinetics; surface tension, surface energy, interfacial tension, and molecular orientation; adsorption; and the water relations of biocolloids.

These lectures are informative and valuable expositions of the fields they cover, suitable material for all students of biology and biochemistry. They are not too technical, and would serve admirably as a foundation for the required understanding of colloidal behavior in physiological biology. GORTNER has

profited by the early contacts which he mentions in his first lecture; he is himself a great teacher and leader in his field. Many students will find help and inspiration in reading these excellent summaries of the progress of research and interpretation in the field of the colloidal state.—C. A. SHULL.

*British Stem and Leaf Fungi (Coelomycetes)*. Vol. II. By W. B. GROVE. Cambridge: at the University Press. New York: The Macmillan Company, 1937. Pp. ix+406. \$6.00.

This second volume completes the author's detailed morphological account of the British fungi belonging to the Coelomycetes. It includes those Sphaeropsidales comprising Sphaerioidae (with colored spores), Nectrioidae, Excipulaceae, Leptostromataceae, and the Melanconiales. The last order is divided into the Hyalospermae and the Phaeospermae.

As in volume I, most of the species treated have been seen and examined microscopically, and the descriptions of the various species are in the main purely morphological. A number of illustrations are given showing spore characteristics and the types of fructifications. There is included a short summary of geographical distribution, and there are indexes (1) of the Ascomycetes to which the coelomycetous fungi have been assigned by various investigators, (2) of the host genera, and (3) of the binomial names of the fungi treated.

The author's aim has been "to set before the English-speaking reader, for the first time in his own language and so far as it is illustrated by the British species of this group, a panoramic view of the skilful structure erected by the inimitable genius of Saccardo some fifty years ago, to include them all in one scheme." This aim he appears to have accomplished in admirable fashion. To all mycologists and plant pathologists who deal with this group the two volumes should be of outstanding value.—J. M. BEAL.

# THE BOTANICAL GAZETTE

June 1938

## GROWTH OF EXCISED ROOTS OF THE TOMATO

WILLIAM J. ROBBINS AND MARY BARTLEY SCHMIDT

(WITH TWENTY FIGURES)

### Introduction

This investigation<sup>1</sup> was undertaken to define the nutritional requirements of the root of a higher plant, *Lycopersicon esculentum* Mill. The underground root of a land plant contains no chlorophyll and grows in darkness. It depends upon the top for the carbohydrate needed in its development, and in this respect its relation to the top is that of a parasite to its host. Is the root dependent upon the green shoot for other essential materials?

ROBBINS (10) initiated a study of this question in 1917, and found it possible to grow excised root tips of corn (*Zea mays* L.) for considerable periods of time in solution cultures under sterile conditions. He and his coworkers (MANEVAL and V. B. WHITE), however, were unable to secure unlimited growth of the roots of this plant in any of the numerous media tested (11, 12, 13, 14, 16, 17).

P. R. WHITE (29) in 1934 reported unlimited growth of excised tomato roots in a medium of mineral salts, cane sugar, and yeast, a medium similar to that originally used by ROBBINS for corn roots. This discovery offered an opportunity to determine the nutritional requirements of the root of one kind of plant, since a medium which permits unlimited growth obviously contains all that the root re-

<sup>1</sup> The writers express their appreciation for aid from research funds of the College of Agriculture, University of Missouri, furnished through Dean F. B. MUMFORD; and assistance given by FREDERICK KAVANAGH and JASPER CLARK.

quires for growth. A study of the growth of excised roots of tomato under sterile conditions was therefore begun in 1935.

Its purposes were: first, to confirm WHITE's report that unlimited growth of excised tomato roots is possible; second, to determine whether each of the three parts of his medium (the mineral salts, cane sugar, and yeast extract) was essential; third, to define as far as possible in what the essential character of these parts of his medium consisted. Particular attention was devoted to the yeast extract, which was the most complex and ill-defined part of the medium used by WHITE.

Each of the three portions was found essential. The mineral salts could not be omitted, nor could the cane sugar nor the yeast be omitted from the medium if growth was to be secured. The essential nature of the yeast was found to be determined by its content of vitamin B<sub>1</sub> or vitamin thiazole, although its ash content (perhaps its nitrogen content also) is of some importance. Other sources of carbon than cane sugar were discovered to be available, and certain inadequacies in the mineral nutrients in WHITE's solution were studied.

It was concluded that the tomato root probably depends upon the top not only for carbohydrates, but for vitamin B<sub>1</sub> (or thiazole). Notes on this investigation have been published from time to time (15, 18, 19, 20), and some of our results have already been confirmed by WHITE (32, 33, 34).

The significance from the standpoint of plant tissue culture of the development of a solution of known constituents in which unlimited growth of excised roots occurs is obvious. It permits a study of the mineral nutrition, nitrogen synthesis, energy relations, growth and development, and other fundamental physiological processes of a portion of a higher plant in a way hitherto impracticable. It is of some interest to note that while animal tissue culture was successfully used for many years before plant tissue culture had developed, a synthetic medium for animal tissue culture is still to be devised.

A review of the literature on the growth of excised roots in sterile culture or of plant tissue culture will not be attempted here. WHITE (30) has recently presented a review of this subject.

### Material and methods

In these experiments excised roots of tomato were grown under sterile conditions in liquid media. The roots came originally from germinated seeds of a pink-fruited variety from Mexico, Ajo de Verrado no. 580, secured through Dr. J. W. LESLEY and Dr. H. L. BLOOD. This tomato is placed in the species *Lycopersicon esculentum* but it has some characteristics of *L. pimpinellifolium*. The fruits were surface sterilized, and seeds were removed from them and

TABLE 1

DATES OF INITIATION OF VARIOUS PASSAGES REFERRED TO IN TEXT

PASSAGE	BEGUN	PASSAGE	BEGUN
Original.....	Sept. 29, 1935	15.....	Nov. 24, 1936
1.....	Oct. 18	16.....	Dec. 26, 27
2.....	Nov. 5	17.....	Jan. 30, 31, 1937
3.....	Nov. 26	18.....	Mar. 5, 6
3a.....	Dec. 7	19.....	Apr. 6
4.....	Dec. 24	20.....	May 8
5.....	Jan. 30, 1936	21.....	June 9
6.....	Feb. 23	22.....	July 16
7.....	Mar. 20	23.....	Aug. 23
8.....	Apr. 11	24.....	Sept. 27
9.....	May 20	25.....	Oct. 19
10.....	June 20	26a.....	Nov. 15
11.....	July 18	26b.....	Nov. 26, 27
12.....	Aug. 20	27a.....	Dec. 14
13.....	Sept. 23	27b.....	Dec. 22
14.....	Oct. 21, 22	27c.....	Jan. 10, 1938

placed on sterile distilled water agar to germinate. After the roots were several centimeters long, 2 mm. tips were cut off and placed in flasks of the basic medium, WHITE's solution (29).

All of the experimental work, unless otherwise noted, was performed with subcultures from two of these original excised roots, B and C. Subcultures were made at approximately monthly intervals resulting up to the present time in a total of twenty-seven passages from the original roots. Approximately 3000 subcultures have been made over a period of about  $2\frac{1}{2}$  years. The dates for the passages are given in table 1.

The tomato root cultures represent two pure lines or clones. The results secured with these two clones did not differ significantly.

In making subcultures, pieces were cut from roots in the liquid

media by means of a fragment of a razor blade mounted in an aluminum handle. Each piece, except in a few special instances, had one or more growing tips. The cutting tool was sterilized by dipping it in 95% alcohol and burning off the alcohol. This prevented overheating of the blade or handle. Pieces of root were transferred to new culture media by means of a loop needle of chromium wire mounted in aluminum. The needles were sterilized in the flame and allowed to cool before touching the root fragment. All work involved in transferring roots was carried out in a steamed culture chamber, and contaminations were not frequent. Whenever a root became contaminated it was discarded.

The excised roots were grown individually in 40 or 50 ml. of solution in 125 ml. Erlenmeyer flasks of Pyrex glass.

The basic medium for these experiments was WHITE's solution (29), which has the following composition:

Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.6	millimols	0.142 gm.
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.3		0.073
KNO <sub>3</sub> .....	0.8		0.081
KCl.....	0.87		0.065
KH <sub>2</sub> PO <sub>4</sub> .....	0.09		0.012
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	0.006		0.0024
Redistilled water.....	1000.00	ml.	
2% by weight of sucrose and 0.01% by weight (100 p.p.m.) of dried brewers' yeast. A filtered extract of the yeast was used.			

WHITE's solution is an adaptation of the modified Pfeffer's solution originally used by ROBBINS (10) with F<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> replacing FeCl<sub>3</sub>.

The sucrose used in preparing WHITE's solution was Pfanstiehl's c.p. sucrose, lot no. 409, except in the earlier experiments in which Merck's highest purity sucrose was used.

The dried brewers' yeast was strain K from Anheuser-Busch, St. Louis, Missouri. The yeast was extracted by boiling in redistilled water for  $\frac{1}{2}$  hour, and centrifuging to bring down the solid material. The supernatant liquid was used.

Merck's synthetic vitamin B<sub>1</sub> (Betabion) was used most frequently. Preparations of the natural crystalline vitamin from Merck and the synthetic vitamin from the Winthrop Universal Co. were used also. No differences in the effects of these various preparations were noted.

Other solutions used frequently in these experiments were solution

W and solution Z. Solution W had the following composition: WHITE's mineral solution, 2% sucrose and 0.1 p.p.m. each of boron and zinc. Solution Z was composed of WHITE's mineral solution, 2% sucrose, and 1 ml. per liter of a modification of Hoagland's A to Z mixture. A modification of Hoagland's A to Z mixture was prepared by adding to 18 liters of water: LiCl 0.5 gm.,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  1.0 gm.,  $\text{ZnSO}_4$  1.0 gm.,  $\text{H}_3\text{BO}_3$  11.0 gm.,  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$  1.0 gm.,  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  0.5 gm.,  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  7.0 gm.,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  1.0 gm.,  $\text{Co}(\text{NO}_3)_2$  1.0 gm.,  $\text{TiSO}_4$  1.8 gm., KI 0.5 gm., NaBr 0.5 gm.

The mineral salts were Merck's highest purity except for the calcium nitrate, which was Merck's chemically pure. Redistilled water was used without exception; the tap distilled water was distilled in Pyrex glassware at least once. All glassware used was Pyrex.

Nutrient media were sterilized in the autoclave at 120° C. for 20 minutes unless otherwise specified. For the first fourteen passages 40 ml. of liquid culture medium and for the remainder 50 ml. of medium per flask were used. Cultures were grown, unless otherwise stated, in diffuse light at room temperature or in water baths with the temperature controlled to some extent.

The effect of different treatments was judged by macroscopic observation at intervals during the growth of each set of cultures. This permitted approximations of rates of growth. Measurements of length were not made because of the profuse branching of the excised roots. Daily growth rates were not determined except on occasion, when sketches were made at intervals to estimate enlargement of fragments.

After about 2 months, those roots which had not been used as inoculum were removed from the culture flasks, rinsed in distilled water and dried. Air dry weights were taken as an indication of total growth. Since the best roots in each set were used for inoculating the next series, the dry weights secured were only an approximation of the total growth and were less than the maximum. All conclusions drawn from the data on dry weights were supported by the observations during the development of any particular set of cultures.

### Experimental work

UNLIMITED GROWTH OF EXCISED TOMATO ROOTS.—Our first concern was to determine the validity of the report by WHITE (29) that



unlimited growth of excised tomato roots is possible. This report was confirmed in these experiments. Excised tomato roots were grown for about  $2\frac{1}{2}$  years through twenty-seven passages in liquid media. The results for twenty passages in WHITE's solution are summarized in table 2. The original tips were placed in WHITE's

TABLE 2  
RECORD OF TWENTY PASSAGES OF EXCISED TOMATO ROOTS  
IN WHITE'S SOLUTION

PASSAGE	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT PER ROOT (MG.)
Original . . . . .	15	34.8	7.0-65.2
1 . . . . .	5	25.9	12.0-39.5
2 . . . . .	10	15.9	1.1-38.5
3 . . . . .	28	17.0	3.0-39.0
4 . . . . .	5	3.0	2.0-3.5
5 . . . . .	15	16.2	2.0-43.0
6 . . . . .	13	11.0	2.0-17.5
7 . . . . .	10	18.5	8.3-33.0
8 . . . . .	8	13.3	4.0-34.0
9 . . . . .	8	28.9	12.6-42.0
10 . . . . .	6	26.0	23.0-32.3
11 . . . . .	7	14.8	7.0-19.5
12 . . . . .	6	22.3	17.0-29.2
13 . . . . .	6	8.5	4.5-11.9
14 . . . . .	15	7.3	4.2-12.1
15 . . . . .	5	7.8	3.3-10.4
16 . . . . .	28	5.4	0.9-10.4
17* . . . . .	3	7.7	2.8-14.1
18 . . . . .	14	14.1	7.7-23.4
19 . . . . .	10	21.4	9.0-28.9
20 . . . . .	18	20.9	18.3-24.2

\* Solution Z plus yeast extract used.

solution September 29, 1935. Transfers were made at approximately monthly intervals; the twentieth passage was made May 8, 1937.

While the data presented in table 2 confirm WHITE's report of the possibility of unlimited growth of excised tomato roots, they also show that considerable variation in growth occurred during the period of the experiments. The original roots in WHITE's solution grew better than those of any subsequent set, even though the root fragments used as inoculum were smaller for the original cultures than those used for later series (fig. 1). This suggests that the root fragments from the seedling root contained material which favored

their growth and which was not supplied in adequate quantity by the constituents of WHITE's solution. There was no indication of a progressive decrease in growth, however; in fact growth in passages 19 and 20 was as great as in passages 1 and 2.

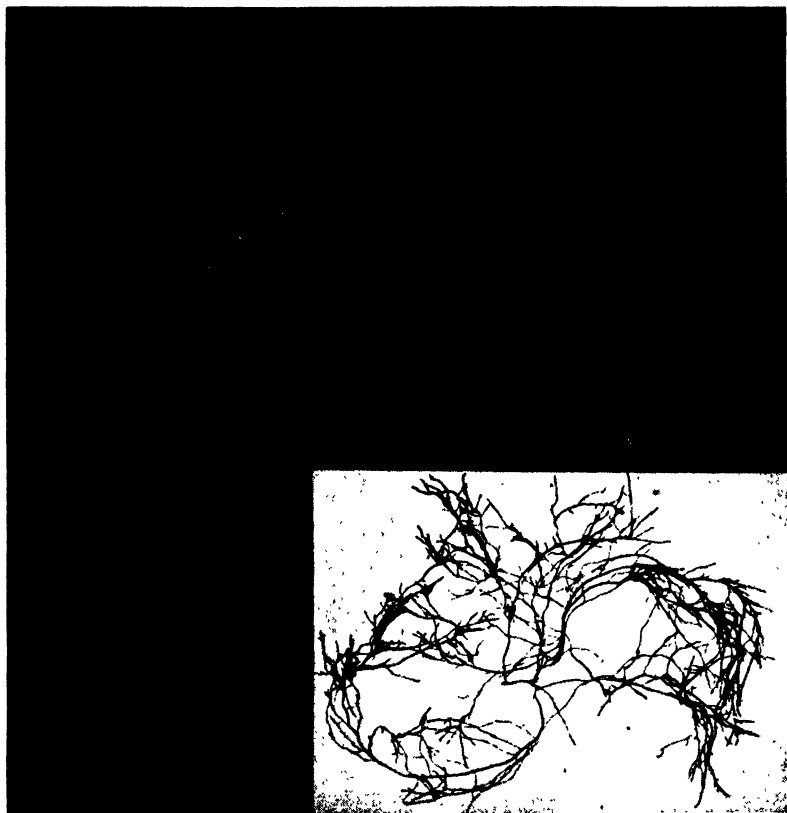


FIG. 1.—Original excised tomato root (above) and root from passage 19 (below). Both grown in WHITE's solution and shown on same scale.

A part of the considerable variation in growth in the successive passages was probably the result of differences in temperature of incubation. For example, the least growth occurred in passages 4 and 16, both of which were started in the month of December. WHITE (31) found a seasonal fluctuation in growth rate which he correlated with seasonal fluctuation in temperature.

In these experiments considerable variation among roots grown under the same conditions in a single passage was noted. This is shown by the range of the dry weights of individual roots for each passage as given in table 2. This variability in growth of roots in a given series grown under uniform conditions was noted throughout the experiments and is discussed later.

**INDISPENSABILITY OF MINERAL SALTS, SUGAR, AND YEAST IN WHITE'S SOLUTION.**—In addition to water and dissolved gases including oxygen, WHITE's solution is composed of three main parts: yeast extract, sugar, and a mixture of mineral salts. Is each of these

TABLE 3  
DATA DEMONSTRATING INDISPENSABILITY OF YEAST, SUGAR  
AND MINERAL SALTS IN WHITE'S MEDIUM

PASSAGE	MODIFICATION OF WHITE'S MEDIUM	No. ROOTS		DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
		USED	WEIGHED		
2.....	None	28	19	15.9	1.1-38.5
2.....	Yeast omitted	20	1	0.2	.....
3.....	None	14	8	10.3	4.0-20.0
3.....	Sugar omitted	9	7	0.04	.....
3.....	Minerals omitted	9	8	0.09	.....
18.....	None	20	14	14.1	7.7-23.4
18.....	Yeast omitted	15	15	0.57	0.2- 1.7

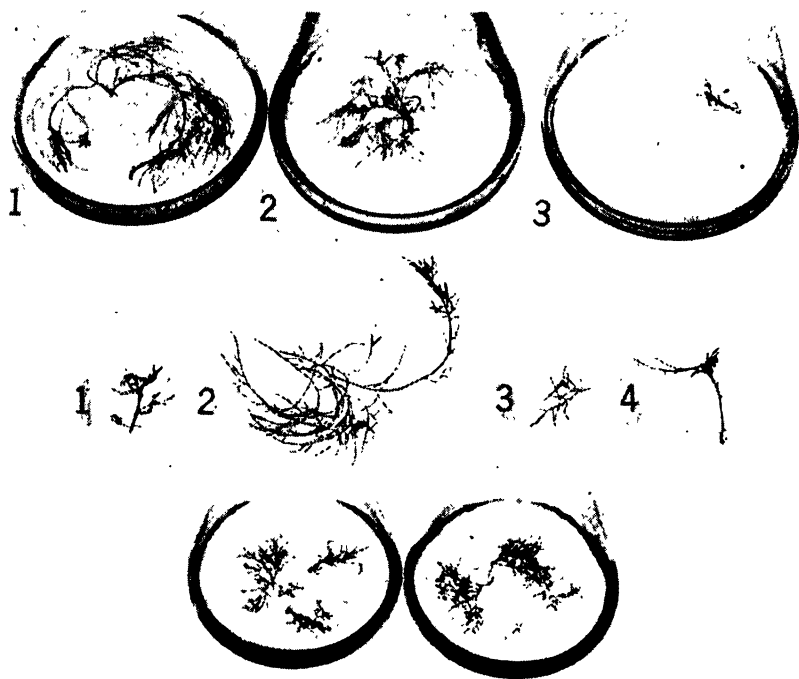
three parts essential for the growth of excised tomato roots? In our experiments little or no growth occurred in WHITE's medium from which any one of these three parts was omitted. The indispensable character of each of these constituents has been reported briefly (18) and confirmed by WHITE (32).

The indispensability of the yeast extract, the sugar, or the mineral salts is shown by the data in table 3. In passage 2, although but one root was weighed, none of the twenty grew appreciably in WHITE's solution with yeast omitted (fig. 2). Some roots grew slightly (maximum weight 1.7 mg.) in the solution lacking yeast in passage 18. This was believed to be at the expense of material carried over in the inoculum. In the absence of minerals or of sugar the growth was inappreciable (table 3).

Each part of the basic medium (WHITE's solution) was essential

for the growth of tomato roots in pure culture. The absence of any of the three portions resulted in complete failure to grow, not merely in a decrease in rate of growth.

**YEAST AND GROWTH OF TOMATO ROOTS.**—It was found in the experiments just described that the yeast in WHITE's solution was in-



FIGS. 2-4.—Fig. 2 (above), effect of absence of yeast extract on growth (passage 18): 1, in WHITE's solution; 2, in same solution with 10 gamma vitamin B<sub>1</sub> instead of yeast; 3, in same solution without yeast or vitamin B<sub>1</sub>. Fig. 3 (center), effect of yeast functions on growth: 1, in WHITE's solution with absolute alcoholic extract of yeast; 2, with 80% alcoholic extract; 3, with subsequent water extract; 4, with ash of residue. Second passage in media used in 1, 2, and 3. Fig. 4 (below), unlimited growth in medium of mineral salts, cane sugar, and vitamin B<sub>1</sub>. Two roots from twelfth passage in this medium.

dispensable for the growth of excised tomato roots. Why is yeast indispensable, and can any other natural or synthetic products be found which will replace the yeast portion of WHITE's solution? From our results it is believed that the indispensable character of the yeast

is because of its vitamin B<sub>1</sub> or thiazole<sup>2</sup> content. This statement does not imply that other constituents of the yeast are not beneficial. We have found, also, that yeast may be replaced in WHITE's solution by other natural products, including malt flour, some samples of maltose, and neo-peptone.

Yeast extract as used in our experiments was a mixture of substances, including mineral salts, nitrogenous compounds, various vitamins, and an unknown number of other organic compounds. There was a possibility that not all of the constituents of the yeast were essential to a satisfactory medium. In order to determine in what the essential nature of the yeast consisted, two procedures appeared possible: (a) fractionating the yeast to discover what portion of the yeast extract was essential; (b) substituting for yeast in WHITE's solution specific compounds known to be present in the yeast. Both methods were used in these investigations.

A. FRACTIONATION OF YEAST.—By successive extraction of yeast with absolute ethyl alcohol, 80% alcohol, and water it was found that the beneficial factor, or factors, were but slightly soluble in absolute alcohol and almost entirely removed by 80% alcohol.

The dried brewers' yeast was fractionated as follows: 1 gm. of dried yeast was mixed with about 100 ml. of absolute alcohol, allowed to stand for 10–15 minutes, mixed thoroughly and filtered through Whatman no. 50 (hardened) filter paper. The filtrate was evaporated to dryness and boiled with 100 ml. of redistilled water. The residue from the absolute alcohol extraction was washed on the filter paper several times with 80% alcohol, and the filtrate, yellow in color, was treated as the absolute alcoholic extract was treated. The residue left by these two extractions was washed several times with hot redistilled water and the filtrate evaporated to 100 ml. The final residue was removed from the filter paper and ashed at low-red heat in a muffle furnace. The ash was suspended in 100 ml. of redistilled water. Each of the four fractions was added to WHITE's solution lacking yeast in amounts equivalent to 100 p.p.m. of yeast.

<sup>2</sup> When the terms thiazole or pyrimidine are used in this paper, 4-methyl-5-hydroxy-ethylthiazole or the 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide is meant. These compounds were used by WILLIAMS and CLINE (37) in synthesizing vitamin B<sub>1</sub>.

The effect of the fractions is illustrated by the data in table 4. In passage 11, begun July 18, 1936, thirteen subcultures were made in WHITE's solution and six in each of the following: WHITE's medium with yeast replaced by the absolute alcoholic extract; by the 80% alcoholic extract; by the subsequent water extract; by the ash of the residue. These roots showed that the medium containing the 80% alcoholic extract was much superior to those containing the other

TABLE 4

DRY WEIGHTS OF ROOTS GROWN IN WHITE'S SOLUTION, AND SAME SOLUTION WITH YEAST REPLACED BY VARIOUS YEAST FRACTIONS. SUBCULTURES OF ROOTS GROWN IN PASSAGE 11 USED FOR PASSAGE 12, EXCEPT FOR THOSE IN MEDIUM CONTAINING ASH

PASSAGE	YEAST FRACTION IN WHITE'S SOLUTION	NO. ROOTS		DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
		GROWN	WEIGHED		
11.....	Usual water extract	13	7	14.8	7.0-19.5
	Absolute alcohol extract	6	3	3.1	2.3-4.0
	80% alcohol extract	6	5	8.5	1.1-11.0
	Subsequent water extract	6	4	5.8	4.0-9.0
	Ash of residue	6	6	1.4	0.2-5.0
12.....	Usual water extract	6	6	22.5	17.0-29.2
	Absolute alcohol extract	6	5	1.1	0.4-2.4
	80% alcohol extract	6	6	8.6	5.3-10.2
	Subsequent water extract	6	6	1.1	0.7-1.9
	Ash of residue	6	5	1.7	0.7-2.2

fractions. Growth in the medium containing the absolute alcoholic extract, or the water extract, was slight; in that containing the ash of the residue almost no growth occurred.

Subcultures were made (passage 12) from the roots grown in the media containing each fraction of the yeast except that containing the ash, in which not enough growth occurred to furnish inoculum. Growth of subcultures in the medium containing the 80% alcoholic extract was comparable with that in passage 11, but almost no growth occurred in media containing the absolute alcoholic extract and the water extract. Typical roots are shown in figure 3.

Growth of the roots in the media containing the various yeast fractions showed that the active principle in yeast could be largely

removed by treatment with 80% alcohol. The 80% alcoholic extract was not so effective as the water extract of yeast customarily used in preparing WHITE's solution. However, enough of the essential portion was removed by alcoholic extraction so that a subsequent water extract was not adequate for continued growth.

B. VITAMIN B<sub>1</sub> AND GROWTH OF TOMATO ROOTS.—Vitamin B<sub>1</sub> is a constituent of dried yeast, is soluble in 80% alcohol and in water, and sparingly soluble in absolute alcohol (18). SCHOPFER (23) demonstrated the essential nature of vitamin B<sub>1</sub> for the growth of a mold, *Phycomyces blakesleeanus* Burgeff. Experiments were therefore initiated October 22, 1936, to determine the effect of this vitamin on the growth of excised tomato roots. These experiments showed that unlimited growth of tomato roots will probably occur in a solution of mineral salts, chemically pure sucrose, and vitamin B<sub>1</sub>. Excised tomato roots have been carried through fourteen passages over a period of more than 14 months in this medium. The effect of the vitamin as used here was not so great as that of the yeast extract; but as a substitute for yeast in WHITE's solution it was adequate for continued growth at a low level. The effect of replacing the yeast in WHITE's solution by vitamin B<sub>1</sub> is shown in the following experiments.

Twenty-seven subcultures were made in WHITE's solution in passage 14, and eleven in the same medium with vitamin B<sub>1</sub>, 10 gamma per flask, replacing the yeast. From these roots subcultures were continued through fourteen passages in a medium of mineral salts, Pfanstiehl's c.p. sucrose no. 409, and vitamin B<sub>1</sub>. The dry weights of roots for twelve of these passages are given in table 5. For each passage the best roots were used as material for inoculating the solutions used in the succeeding passage. The dry weights given in table 5 do not therefore equal the maximum average dry weight per root for each passage. From these data, as well as observations made during the period covered by the experiments, it appears that unlimited growth of excised tomato roots is possible in a medium of mineral salts, cane sugar, and vitamin B<sub>1</sub>. Figure 4 shows two roots from the twelfth successive passage in this medium. There was no constant decrease in growth as would be anticipated were this medium inadequate. Variations in growth occurred, due in part to differences

in incubation temperatures. For example, the temperatures in passages 22 to 24 were lower than those for the other passages. The variations were also the result in part of differences in the mineral salts used. For example, in passages 20 to 25, the salts of WHITE's solution were supplemented in the solutions containing vitamin B<sub>1</sub> by the addition of 0.1 p.p.m. of B as boric acid and 0.1 p.p.m. of Zn as zinc sulphate. These supplements and a higher temperature im-

TABLE 5

GROWTH OF ROOTS IN SUCCESSIVE PASSAGES IN WHITE'S SOLUTION, AND  
IN SAME SOLUTION WITH YEAST REPLACED BY VITAMIN B<sub>1</sub>  
10 GAMMA PER FLASK

PASSAGE	WHITE'S SOLUTION		WHITE'S SOLUTION WITH VITAMIN B <sub>1</sub> REPLACING YEAST		
	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
14.....	9	6.7	8	4.5	0.8-7.3
15.....	5	7.8	7	7.1	2.7-17.1
16.....	28	5.4	5	4.6	1.6-5.5
17.....	3	7.7	7	4.0	1.4-5.6
18.....	14	14.1	7	4.4	0.9-10.4
19.....	10	21.4	9	7.5	2.6-23.1
20*.....	4	22.0	14	11.5	1.9-26.7
21*.....	(not used)	.....	6	16.5	10.7-25.4
22*†.....	(not used)	.....	6	7.5	5.2-8.2
23*†.....	(not used)	.....	7	5.6	0.4-9.6
24*†.....	(not used)	.....	5	3.0	2.1-5.4
25*.....	3	8.7	4	8.3	6.0-11.2

\* 0.1 p.p.m. boron and 0.1 p.p.m. zinc added to medium containing vitamin B<sub>1</sub>.

† Roots grown at about 16° C. (others grown at room temperature).

proved the growth, as shown by the dry weights in passages 20 and 21. Nevertheless, the growth in WHITE's solution was superior to that in the same solution with vitamin B<sub>1</sub> replacing the yeast. This is discussed in more detail later.

C. AUTOCLAVED YEAST, AUTOCLAVED VITAMIN B<sub>1</sub>, AND GROWTH OF TOMATO ROOTS.—The solutions in which yeast extract or vitamin B<sub>1</sub> was used in these experiments were heated for 20 minutes at 120°C. It was assumed that this period of heating in an acid solution (pH 5.0 to 5.2) did not destroy the vitamin. Furthermore we used vita-



min B<sub>1</sub> which had been filtered sterile and secured results similar to those obtained with the vitamin solutions which had been sterilized by heating. However, vitamin B<sub>1</sub> in alkaline solution is largely destroyed by long continued high temperatures (36). If vitamin B<sub>1</sub> were the constituent of yeast extract essential for the growth of tomato roots, then the growth promoting effect should be destroyed by sufficient heating in alkaline suspension; and vitamin B<sub>1</sub> autoclaved in alkaline solution should lose its growth promoting

TABLE 6

DRY WEIGHTS OF ROOTS GROWN IN WHITE'S SOLUTION AND IN SAME SOLUTION WITH USUAL YEAST EXTRACT REPLACED BY YEAST EXTRACT FROM: YEAST HEATED AT 120° C. FOR 5 HOURS OR FOR 12 HOURS AT INITIAL PH 8.9, BY VITAMIN B<sub>1</sub>, OR BY VITAMIN B<sub>1</sub> HEATED AT 120° C. FOR 12 HOURS AT INITIAL PH 8.9

PASSAGE	SPECIAL TREATMENT OF WHITE'S SOLUTION	NO. ROOTS		DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
		USED	WEIGHED		
15.....	(None	30	5	7.8	3.3-10.4
	(Yeast, 120° C. for 5 hours	10	7	10.3	3.8-15.4
16.....	(None	40	28	5.4	0.9-10.4
	(Yeast, 120° C. for 12 hours	10	9	14.3	9.3-19.9
	(Vitamin B <sub>1</sub>	10	5	4.6	1.6- 5.5
	(Vitamin B <sub>1</sub> , 120° C. for 12 hours	10	9	4.7	0.7- 9.5

properties. In our experiments, however, the effectiveness of yeast autoclaved at 120° C. for 5 hours at initial pH 8.0 and at 120° C. for 12 hours at the same initial pH was increased. Furthermore vitamin B<sub>1</sub> autoclaved 12 hours at 120° C. at initial pH 8.0 was still active.

The effect of heating yeast or vitamin B<sub>1</sub> is illustrated in table 6. The yeast suspension from which the extract was made in passage 15 was heated for 5 hours at 120° C. and in passage 16 for 12 hours at 120° C. The vitamin was heated for 12 hours at 120° C. in passage 16. In each instance the original reaction of the yeast suspension or the vitamin solution was adjusted to pH 8.9 before heating. At the end of the period of heating, however, the reaction had returned to approximately its original value of pH 5.1.

In both passages the extract made from the yeast which had been autoclaved for several hours was superior in its effect to that which had been prepared by boiling. Furthermore the effect of the yeast which had been heated for the longer period was somewhat greater (table 6). The greater effect of the yeast extract which had been heated for several hours as compared with that prepared as usual by boiling for a few minutes might be the result of destroying some injurious materials in the extract or of producing some beneficial decomposition products. We are inclined to believe, however, that it is probably associated with the more complete extraction from the yeast of substances which may be inorganic in character. This hypothesis is favored because of our conclusion, presented later, that the mineral salts in WHITE's solution are not adequate for the best growth of tomato roots, at least as we have used it.

In any event these experiments suggested that other substances might replace vitamin B<sub>1</sub> in the growth of tomato roots.

D. INTERMEDIATES OF VITAMIN B<sub>1</sub> AND GROWTH OF TOMATO ROOTS.—The failure of prolonged autoclaving in alkaline solution to destroy the growth promoting effects of yeast or of vitamin B<sub>1</sub> raised the question as to whether other substances replace vitamin B<sub>1</sub> in the growth of excised tomato roots.

WILLIAMS and CLINE (37) synthesized vitamin B<sub>1</sub> from a pyrimidine and a thiazole. Through the courtesy of Dr. R. R. WILLIAMS and of Merck and Co., samples of 4-methyl-5-hydroxy-ethylthiazole, of 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide, and of 2-methyl-5-ethoxymethyl-6-aminopyrimidine were secured. It was found that for the growth of tomato roots vitamin B<sub>1</sub> could be replaced by the two intermediates or by the thiazole alone. This was demonstrated by the following experiments.

In passage 19 (begun April 11, 1937) six transfers were made to solution W and five transfers to solution W, with each of the following supplements: 5 gamma of thiazole per flask, 5 gamma of ethoxypyrimidine per flask, 5 gamma of bromopyrimidine per flask, 5 gamma each of ethoxypyrimidine and thiazole, 5 gamma each of bromopyrimidine and thiazole, and 5 gamma of vitamin B<sub>1</sub>. Growth occurred in all cultures which contained either vitamin B<sub>1</sub> or thiazole. No growth occurred in solution W, nor in the same solutions

supplemented with either the ethoxypyrimidine or the bromopyrimidine. Roots grew in the solutions supplemented with vitamin B<sub>1</sub>, thiazole, and with the mixtures of thiazole and either pyrimidine (table 7). Growth in the mixture of thiazole and pyrimidine showed that the failure of roots to grow in the solutions supplemented with the pyrimidines alone was not because of the toxicity of the latter solutions. All roots were used in the dry weight determinations except the best root in the thiazole series, which was used as

TABLE 7

DRY WEIGHTS OF ROOTS FROM PASSAGE 19 GROWN IN SOLUTION W AND IN SAME SOLUTION WITH VITAMIN B<sub>1</sub> OR INTERMEDIATES OF VITAMIN B<sub>1</sub>

ADDITIONS TO SOLUTION W	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
None.....	6	0.6	0.3- 0.7
Vitamin B <sub>1</sub> .....	5	6.95	2.0-17.3
Thiazole.....	4	3.6	1.2- 8.2
Thiazole and ethoxypyrimidine.....	5	4.0	2.0- 8.1
Thiazole and bromopyrimidine.....	5	4.3	2.1-13.5
Ethoxypyrimidine.....	5	0.8	0.4- 1.6
Bromopyrimidine.....	5	0.7	0.2- 1.5

an inoculum for the subsequent transfer to solutions containing thiazole.

Since the original experiment with thiazole, the tomato roots have been maintained in solution W plus thiazole through eight successive passages extending over 8 months. The roots in the solution containing mineral salts, cane sugar, and thiazole give every indication that unlimited growth of excised tomato roots is possible in this medium. In fact (table 8) the growth in the solutions supplemented with thiazole alone appeared to be somewhat superior to that in the solution supplemented with vitamin B<sub>1</sub> (fig. 5).

We are not prepared to explain the apparent superiority of the thiazole at 10 gamma per flask over the vitamin B<sub>1</sub>. It may have been an effect of some beneficial impurity in the thiazole or the fact that the vitamin B<sub>1</sub> and the thiazole were not used in molecularly equivalent quantities. In any event the important finding was the

apparent possibility of unlimited growth in a solution consisting of mineral salts, cane sugar, and the vitamin thiazole.

The growth of excised tomato roots in solutions with thiazole replacing the yeast was characteristic. Various other solutions gave other characteristic types of growth; these will be discussed in more detail later. Figure 5 shows a typical root in solution W plus thiazole; the branching was profuse, with the main branches long and the secondary branches somewhat short. This root differs markedly

TABLE 8

DRY WEIGHTS OF ROOTS THROUGH SEVEN PASSAGES IN SOLUTION W WITH  
10 GAMMA OF VITAMIN B<sub>1</sub> PER FLASK, AND IN SAME SOLUTION  
WITH 10 GAMMA OF THIAZOLE PER FLASK

PASSAGE	SOLUTION W PLUS VITAMIN B <sub>1</sub>		SOLUTION W PLUS THIAZOLE		
	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
19*	5	7.0	4	3.6	1.2-8.2
20.....	14	11.5	4	13.7	.....
21.....	6	16.5	2	24.6	23.8-25.5
22†.....	6	7.5	2	12.9	11.7-14.1
23†.....	7	5.6	4	17.3	13.7-21.8
24†.....	5	3.0	4	23.1	14.3-32.9
25.....	4	8.3	6	11.9	8.1-14.5

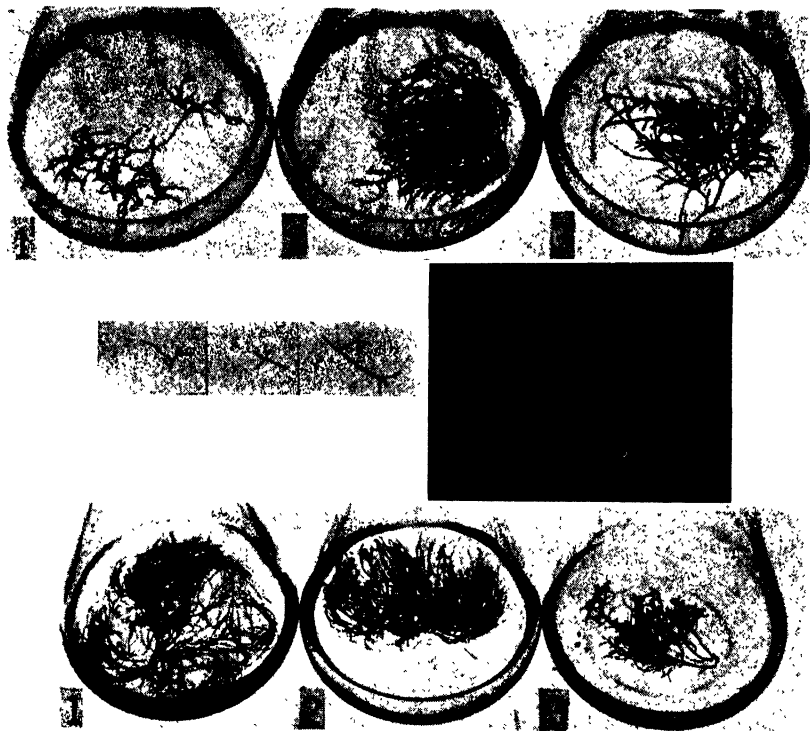
\* Five gamma each of supplements used.

† Roots kept at approximately 16° C. (others grown at room temperature).

from the other two roots shown in figure 5. The root in solution W plus B<sub>1</sub> is also typical, and different in appearance from the thiazole root. It should be noted that one of these two solutions contained 0.2 gamma of vitamin B<sub>1</sub> per ml. and the other 0.2 gamma of thiazole.

Does the tomato root require thiazole alone or does it require vitamin B<sub>1</sub>, synthesizing under the conditions of our experiments sufficient pyrimidine for growth but an inadequate amount of thiazole? We are of the opinion that the tomato root requires the vitamin and synthesizes the necessary pyrimidine but forms little or no thiazole. This assumption is supported by the fact that tomato roots grown in a solution supplemented with thiazole alone form pyrimidine.

E. SYNTHESIS OF PYRIMIDINE BY TOMATO ROOTS.—The synthesis by excised tomato roots of a pyrimidine which will form a portion of the vitamin B<sub>1</sub> molecule was demonstrated by the use of *Phycomyces blakesleeanus*. This fungus requires for growth an external supply of



FIGS. 5-7.—Fig. 5 (above), thiazole and growth of tomato roots (passage 23): 1, solution W plus vitamin B<sub>1</sub>; 2, WHITE's solution with Eastman's maltose instead of cane sugar; 3, solution W plus thiazole. Fig. 6 (center), effect of indole(3)acetic acid in dark. Left to right: in WHITE's solution plus 1 p.p.m. acid; plus 0.1 p.p.m.; plus 0.01 p.p.m.; plus 0.001 p.p.m. Fig. 7 (below), effect of malt flour; WHITE's medium with 100 p.p.m. extract of malt flour instead of yeast: 1, fresh extract added four times; 2, solution replaced four times; 3, undisturbed.

vitamin B<sub>1</sub> or of both intermediates. One intermediate (either the pyrimidine or thiazole alone) is inadequate (21). Tomato roots were grown in solution W supplemented with 10 gamma of vitamin thiazole per flask. At the end of approximately 1 month of growth the roots were removed from the solutions, dried, and weighed. By

suitable additions the solutions in which the roots had grown were adjusted to contain 0.5%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.5%  $\text{KH}_2\text{PO}_4$ , 1% asparagine, and 10% dextrose. The solutions were sterilized and inoculated with *Phycomyces*. A small amount of submerged mycelium developed, the dry weight of which indicated the presence in the solution in which a tomato root had grown of between 0.003 and 0.015 gamma of pyrimidine. The dried roots were powdered and added to a solution of the composition just given. The solutions were sterilized and inoculated with *Phycomyces*. The resulting growth indicated the presence in each root of between 0.3 and 1.0 gamma of vitamin pyrimidine. It appears therefore that tomato roots grown in a solution of mineral salts, sugar, and vitamin thiazole synthesize a pyrimidine which will form a portion of vitamin  $\text{B}_1$ .

F. SUBSTITUTES FOR YEAST OTHER THAN VITAMIN  $\text{B}_1$  AND ITS INTERMEDIATES.—The experiments show that the indispensable character of the yeast is probably related to its content of vitamin  $\text{B}_1$  or intermediates, especially thiazole. We have been interested in determining whether any other specific compounds or natural products would replace the yeast. No specific compound other than vitamin  $\text{B}_1$  or thiazole was found. However, malt flour, maltose, and peptone were used successfully as substitutes for yeast.

(a) INDOLE(3)ACETIC ACID.—Concentrations of 1, 5, and 15 p.p.m. were used as a substitute for the yeast extract in WHITE's solution. Growth in the solutions containing indole(3)acetic acid was inappreciable. While this experiment did not suggest that the acid would replace yeast, it is possible that it was not used at sufficient dilution since stimulation of root growth has been reported at greater dilutions than were used here (4, 7, 26).

Indole(3)acetic acid was used also as a supplement to WHITE's solution. It completely inhibited the growth of excised tomato roots in the dark at concentrations of 1, 0.1, and 0.01 p.p.m. No effect was apparent at a concentration of 0.001 p.p.m. (fig. 6). In the light almost no growth occurred in WHITE's solution containing 15 or 5 p.p.m.; about 50% reduction in growth was produced by 1, 0.1, or 0.01 p.p.m. Growth in the light at concentrations of indole(3)-acetic acid which completely inhibited growth in the dark was because light destroyed the acid. Root fragments grew normally in

WHITE's solution containing 1 p.p.m. of indole(3)acetic acid which had stood in the light for 1 month under sterile conditions. This concentration completely inhibited growth in the dark, and when used a day or two after preparation reduced growth in the light 50% or more.

(b) YEAST ASH.—The ash of an amount of yeast equivalent to that customarily used in WHITE's medium was not found to substitute for the yeast.

(c) FILTER PAPER.—ROBBINS and WHITE (16) found filter paper to be beneficial to the growth of excised corn roots, but it did not substitute for yeast in WHITE's solution. In our experiments  $\frac{1}{2}$  sheet of A. H. Thomas qualitative filter paper was used per flask in place of the yeast extract.

(d) VITAMIN C OR ASCORBIC ACID.—Vitamin C was used at a concentration of 10 gamma per flask in place of the yeast extract in WHITE's solution. It did not substitute for yeast in this medium.

(e) PANTOTHENIC ACID.—Pantothenic acid was used without success as a substitute for the yeast in WHITE's solution. Two samples of the acid were secured from R. J. WILLIAMS, who had found this substance beneficial to certain strains of yeast (35). In passage 16, these two samples were used at 10 gamma per flask in WHITE's medium without yeast, in two sets of ten subcultures each. The roots grew to some extent, but no appreciable growth was obtained when subcultures were made from the best roots in these two sets into more of the same media in passage 17.

(f) LACTOFLAVIN, VITAMIN B<sub>2</sub>, OR VITAMIN G.—Lactoflavin was used without success as a substitute for yeast. In passage 17, ten subcultures were made in solution Z plus 10 gamma of lactoflavin per flask and ten in the same solution supplemented with vitamin B<sub>1</sub>.<sup>3</sup> The roots in the solution with lactoflavin alone did not grow; those with lactoflavin and B<sub>1</sub> grew as well as roots in solution Z plus vitamin B<sub>1</sub>. The failure of lactoflavin to substitute for yeast was not related to an injury caused by the lactoflavin.

(g) ASPARAGINE.—Asparagine at 100 p.p.m. did not substitute for the yeast extract. In passage 17, ten subcultures were made in

<sup>3</sup> Whenever a specific concentration of vitamin B<sub>1</sub> is not stated, the concentration used was 10 gamma per flask or 0.2 gamma per ml.

solution Z plus 100 p.p.m. asparagine, and ten in the same solution supplemented with vitamin B<sub>1</sub>. Abnormal browning of the roots in the medium containing both asparagine and vitamin B<sub>1</sub> indicated that this amount was slightly injurious.

(h) CYSTEINE HYDROCHLORIDE.—Cysteine hydrochloride, considered a growth stimulant by SCHEITTERER (22) and by GAUTHERET (6), did not substitute for the yeast. In passage 17, ten root fragments were cultivated in solution Z plus 10 gamma of cysteine hydrochloride per flask, and ten in the same solution plus vitamin B<sub>1</sub>. Roots in the solution with cysteine hydrochloride alone did not grow; those in the solution containing vitamin B<sub>1</sub> in addition grew appreciably.

(i) INOSITOL.—Inositol (inosite) has been found to be favorable for the growth of yeast (35). Used at a concentration of 100 p.p.m., inositol proved a partial substitute for yeast although the growth obtained was not so good as that secured with 10 gamma of vitamin B<sub>1</sub> per flask. The possibility that the inositol contained vitamin B<sub>1</sub> or thiazole as a contaminant was not investigated, and no subcultures were made.

(j) UREA.—Urea used at 100 p.p.m. injured the excised tomato roots. Root fragments transferred to solution Z containing urea and vitamin B<sub>1</sub> did not grow. In this same solution without urea appreciable growth occurred.

(k) PIMELIC ACID.—Pimelic acid, found to be a growth substance for bacteria by MUELLER (8), was used unsuccessfully as a substitute for yeast. Pimelic acid at 10 gamma per flask and pimelic acid plus vitamin B<sub>1</sub> were used as a substitute for the yeast extract. No growth occurred with the pimelic acid but normal growth was secured with the mixture of pimelic acid and vitamin.

(l) NUCLEIC ACID.—An acid hydrolysate of nucleic acid at 10 gamma per flask proved unsatisfactory as a substitute for the yeast extract. The growth in a medium containing thiazole or vitamin B<sub>1</sub> and the nucleic acid hydrolysate instead of yeast extract was not quite so good as in a medium containing vitamin B<sub>1</sub>. While the nucleic acid hydrolysate was somewhat injurious, failure of growth in the medium in which it replaced the yeast was evidently not the result of the toxicity of the nucleic acid hydrolysate.



(m) MERCK MALTOSE NO. 22068.—In the study of the availability of various sources of carbon, which is discussed later, several samples of maltose were used. Some of them proved to substitute for yeast in addition to acting as a source of carbon. Growth in the solutions in which maltose was substituted for cane sugar and yeast was not so great as in WHITE's solution. Not all samples of maltose, even from the same company, were equally effective; some had no effect and others proved actually injurious. It is assumed that the active maltose contained either vitamin B<sub>1</sub> or a substitute for the vitamin, a finding which is not surprising in view of the results secured by SCHOPFER (23) on the growth of *Phycomyces* in maltose solutions.

Contrary to our expectations, however, the beneficial material in maltose was largely removed by treatment with Fuller's earth but not by charcoal. This may indicate that the beneficial material was neither vitamin B<sub>1</sub> nor thiazole, since we would anticipate that these substances would be adsorbed by both adsorbents (25); it may have been the result of the procedure followed in using the adsorbents.

An active maltose was treated with Fuller's earth or Merck's medicinal charcoal (4 gm. of the adsorbent to 10 gm. of sugar in solution). The untreated maltose or the treated maltose was used to replace the yeast and cane sugar in WHITE's solution. In addition a water extract of the charcoal was added to the medium containing the untreated maltose; the untreated maltose at 100 p.p.m. was also used to replace the yeast in WHITE's medium. Some growth occurred in the solution containing 2% maltose instead of cane sugar and yeast, indicating the presence of vitamin B<sub>1</sub>, thiazole, or a substitute therefor. The amount of the growth substance in the maltose was considerably less than in the yeast extract, since no growth occurred in the solutions with yeast replaced by 100 p.p.m. of maltose. The treatment with Fuller's earth removed the beneficial material but the addition of vitamin B<sub>1</sub> to the treated maltose permitted growth to occur. This suggests that the effect of the Fuller's earth was to remove vitamin B<sub>1</sub> from the maltose. Treatment of the maltose with charcoal improved growth somewhat, however, although still further improvement was noted when vitamin B<sub>1</sub> was added to the maltose treated with charcoal. Either the active material in the maltose was not vitamin B<sub>1</sub> (or thiazole) or the treatment with charcoal for some reason did not remove it as anticipated.

(n) **MALT FLOUR.**—The favorable effects observed with some samples of maltose suggested the use of malt flour as a substitute for yeast. At suitable concentrations it was found to be superior to the yeast extract. The malt flour was prepared by crushing malt in a press under about 12,000 pounds pressure and collecting the flour which could be rubbed through a fine sieve. Malt flour extract was prepared as was the yeast extract; the flour was boiled for approximately one-half hour in redistilled water; the suspension was centrifuged and sufficient of the supernatant liquid was used to be equivalent to 100 p.p.m. of the malt flour in the culture solution.

TABLE 9

DRY WEIGHTS OF ROOTS FROM PASSAGE 18, GROWN IN WHITE'S  
SOLUTION WITH WATER EXTRACT OF 100 P.P.M. MALT FLOUR  
REPLACING YEAST EXTRACT

TREATMENT	AGE OF ROOTS (MONTHS)	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)
Undisturbed.....	2	1	11.4
Solution replaced four times .....	2	1	30.2
Malt flour extract added four times .....	2	1	35.0
Undisturbed.....	4	2	15.2
Solution replaced four times .....	4	2	52.2
Malt flour extract added four times .....	4	2	71.0

The excised roots grew rapidly for about 10 days in the medium containing an extract of malt flour and then ceased growth. This suggested that something in the flour was exhausted by the roots, resulting in a cessation of growth. This assumption was substantiated by noting the beneficial effects of adding fresh quantities of malt flour to a solution in which growth had ceased and by replacing the solution with a fresh solution containing extract of the flour. These results are illustrated by the data in table 9 and by figure 7.

Fifteen subcultures were made in WHITE'S medium with 100 p.p.m. of extract of malt flour in place of the yeast. Five of these subcultures were left undisturbed; for five the solution was poured off and fresh solution added four times at intervals of about 2 weeks; and to five, fresh malt flour extract was added four times at 2 week intervals. At the end of 2 months the dry weight of a root from those solutions receiving fresh supplies of malt flour was about

three times that of a root which had had but one lot of malt flour. The difference at the end of 4 months was still greater (table 9).

The material in the malt flour which limits the growth of the tomato roots appeared to be vitamin B<sub>1</sub> or a substitute therefor. This followed because the addition of vitamin B<sub>1</sub> to malt flour markedly improved growth, and because treatment of malt flour

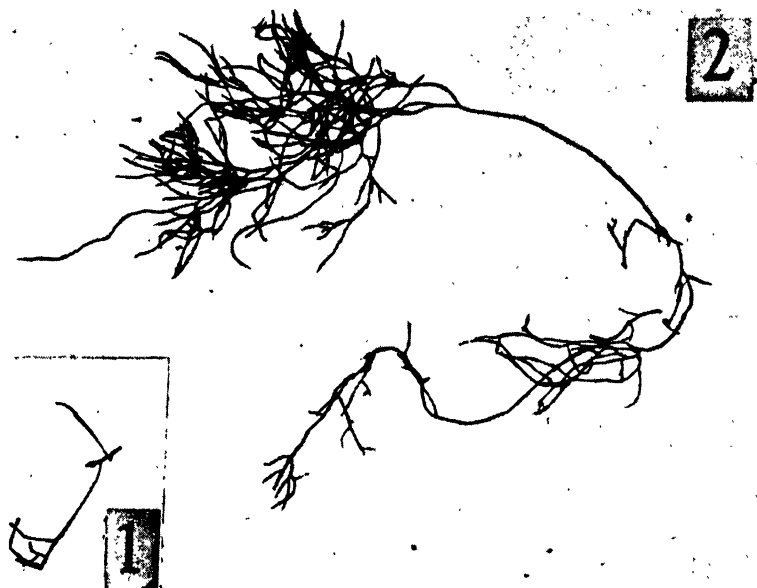


FIG. 8.—Effect of treatment of malt flour with charcoal: 1, in WHITE's solution with yeast replaced by extract of malt flour treated with charcoal; 2, in same solution plus vitamin B<sub>1</sub>.

with charcoal removed the active material. The addition of vitamin B<sub>1</sub> to cultures containing malt flour treated with charcoal permitted some growth, although apparently other beneficial materials also were removed by the charcoal (fig. 8).

(o) PEPTONE.—ROBBINS (11) found peptone favorable for the growth of excised corn roots. We found that at a sufficiently high concentration peptone was a substitute for yeast for the growth of tomato roots. Neo-peptone (Digestive Ferments Co.) was used at two concentrations, 10 and 100 p.p.m., as a substitute for yeast.

Growth of excised tomato roots occurred in the solutions in which 100 p.p.m. peptone replaced the yeast, but very little growth occurred with the 10 p.p.m. peptone (table 10 and figure 9). Since the addition of vitamin B<sub>1</sub> to the solution containing 10 p.p.m. peptone, but no yeast, markedly improved growth, we believe that the vitamin B<sub>1</sub> (or thiazole) content of the peptone was the limiting factor in determining growth in the presence of peptone. It is estimated that the 100 p.p.m. of peptone supplied 0.001 gamma or less of vitamin

TABLE 10

DRY WEIGHTS OF ROOTS FROM PASSAGE 24 IN SOLUTION W WITH PEPTONE, PEPTONE AND VITAMIN B<sub>1</sub>, OR THIAZOLE. ALL ROOTS TRANSFERRED FROM EASTMAN'S MALTOSE CULTURES EXCEPT THOSE IN THIAZOLE, WHICH WERE IN SEVENTH PASSAGE IN THIS SOLUTION

ADDITIONS TO SOLUTION W	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
Peptone 10 p.p.m.....	5	1.3	0.6-1.6
Peptone 10 p.p.m. and vitamin B <sub>1</sub> ..	5	10.8	8.2-12.8
Peptone 100 p.p.m.....	5	7.6	5.0-8.8
Peptone 100 p.p.m. and vitamin B <sub>1</sub> ..	5	18.3	15.2-20.6
Thiazole.....	4	23.1	14.3-32.9

B<sub>1</sub>; that is, the vitamin B<sub>1</sub> content of peptone, judging from our results, was 0.00002% or less.

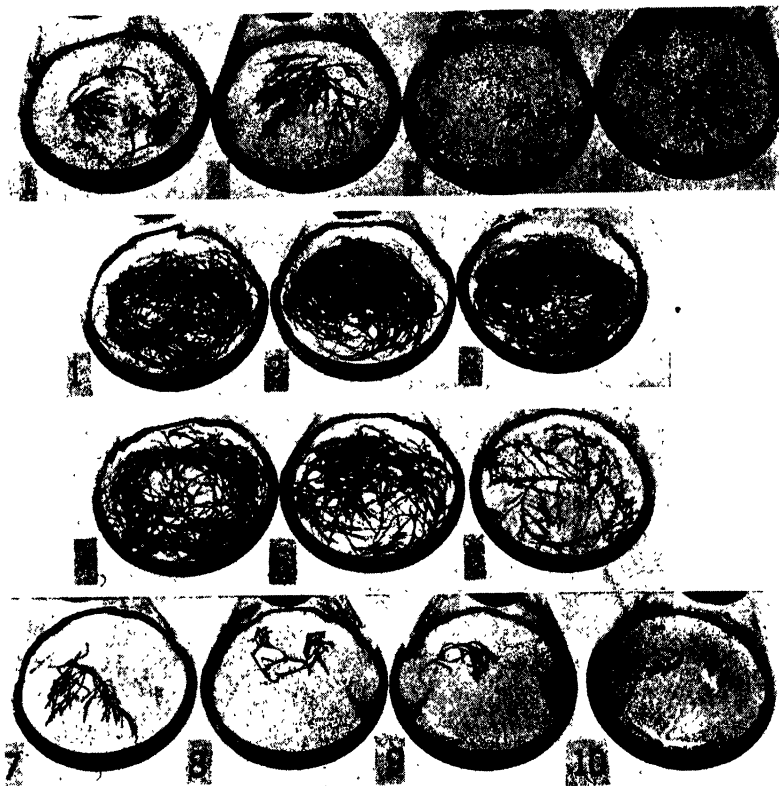
#### CONCENTRATION OF VITAMIN B<sub>1</sub> AND INTERMEDIATES

Since vitamin B<sub>1</sub> or thiazole was found to substitute for yeast in permitting unlimited growth of excised tomato roots, it was considered advisable to determine the effect of dilution of the vitamin and of its intermediates. Various dilutions were used in WHITE's solution lacking yeast, in solution W, and in WHITE's solution with cane sugar and yeast replaced by a light brown commercial sugar, or by the same sugar treated with charcoal.

In passage 20, WHITE's solution was used with the cane sugar and yeast replaced by light brown sugar and vitamin B<sub>1</sub>. Quantities of the vitamin from 100 gamma to 0.000001 gamma per flask containing 50 ml. of solution were used. The results of the experiment,

which ran for 9 weeks, are given in table 11 and photographs of typical roots are shown in figure 10.

A definite, although small, effect of 0.000001 gamma of vitamin B<sub>1</sub> was observed. The roots in flasks containing this quantity of the



FIGS. 9, 10.—Fig. 9 (above), effect of peptone (passage 24): 1, in solution W plus 100 p.p.m. peptone; 2, in same solution plus 10 gamma vitamin B<sub>1</sub>; 3, in solution W plus 10 p.p.m. peptone; 4, in same solution plus 10 gamma vitamin B<sub>1</sub>. Fig. 10 (below), effect of different amounts of vitamin B<sub>1</sub> (passage 20). Growth in WHITE's solution with yeast and cane sugar replaced by light brown commercial sugar plus: 1, 100 gamma vitamin B<sub>1</sub>; 2, 10 gamma; 3, 1 gamma; 4, 0.1; 5, 0.01; 6, 0.001; 7, 0.0001; 8, 0.00001; 9, 0.000001; 10, none.

vitamin averaged 3.6 mg., while those in the solutions containing no vitamin averaged 1.5 mg. The dilution of the vitamin for these solutions was 1 in 40,000,000,000,000. Dividing the increase in dry

weight (2.1 mg.) by the amount of vitamin involved, it is found that in the flasks containing 0.000001 gamma of vitamin 1 part of the vitamin was concerned in producing over 2,000,000,000 parts of dry matter. So far as we are aware, a physiological effectiveness as great as indicated by these figures has not previously been reported.

The maximum effectiveness of the vitamin in this medium occurred at about 0.1 gamma per flask. Further increases of the vitamin up to 100 gamma gave no consistent increase in dry weight.

TABLE 11

AVERAGE DRY WEIGHTS OF ROOTS FROM PASSAGE 20, GROWN IN WHITE'S MINERAL SOLUTION AND 2% LIGHT BROWN SUGAR WITH VARIOUS CONCENTRATIONS OF VITAMIN B<sub>1</sub> OR OF THIAZOLE

AMOUNT SUPPLIED IN GAMMA PER FLASK	VITAMIN B <sub>1</sub>		THIAZOLE	
	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)
100.0.....	2	73.4	4	30.4
10.0.....	3	55.8	4	18.0
1.0.....	2	82.4	4	9.2
0.1.....	4	72.3	4	4.8
0.01.....	4	57.3	4	3.4
0.001.....	4	18.1	.....	.....
0.0001.....	4	5.0	.....	.....
0.00001.....	4	4.2	.....	.....
0.000001.....	4	3.6	.....	.....
None.....	4	1.5	4	1.5

This is clear from the curve in figure 11. Evidently some factor other than the amount of vitamin B<sub>1</sub> limited the growth at the higher concentrations. It was noted that the growth of the individual roots at a given concentration of vitamin B<sub>1</sub> was quite uniform up to that of those in solutions containing 1 gamma of the vitamin. At 1, 10, and 100 gamma, considerable variation occurred and the best roots only were used in the dry weights given in table 11. The variation, which we are not prepared to explain, may be illustrated as follows: In the solution containing 1 gamma per flask two roots averaged 82.4 mg. and two others 27.2 mg.; at 10 gamma per flask three roots averaged 55.8 mg. and one weighed 19.5 mg.; at 100 gamma two roots averaged 73.4 mg. and two others 39.9 mg.

Amounts of thiazole ranging from 0.01 to 100 gamma per flask were used in the medium with light brown sugar. The growth in the thiazole cultures was not so good as in the corresponding cultures containing vitamin B<sub>1</sub>. This is evident from the data in table 11, the photographs in figure 12, and the curves in figure 11. However, a definite effect from 0.01 gamma of thiazole was observed (3.4 mg. of dry matter as compared with 1.5 mg. with no thiazole), and in-

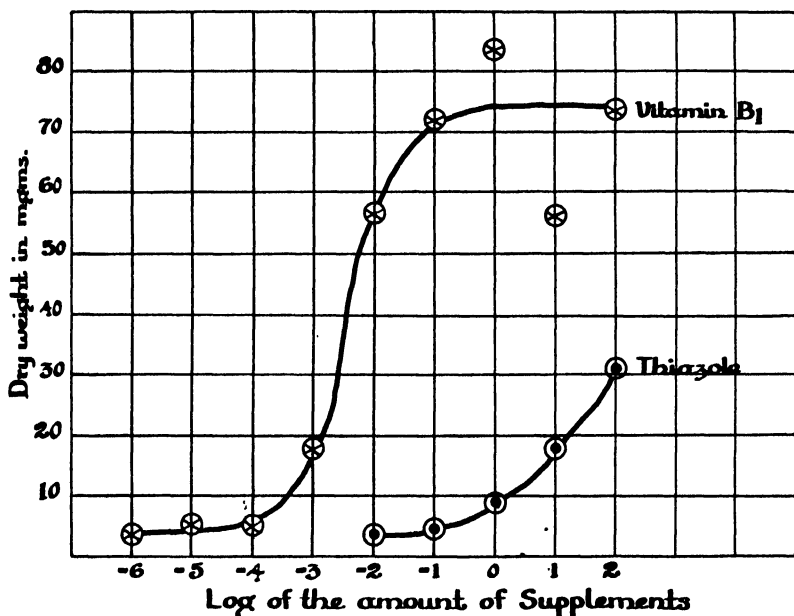


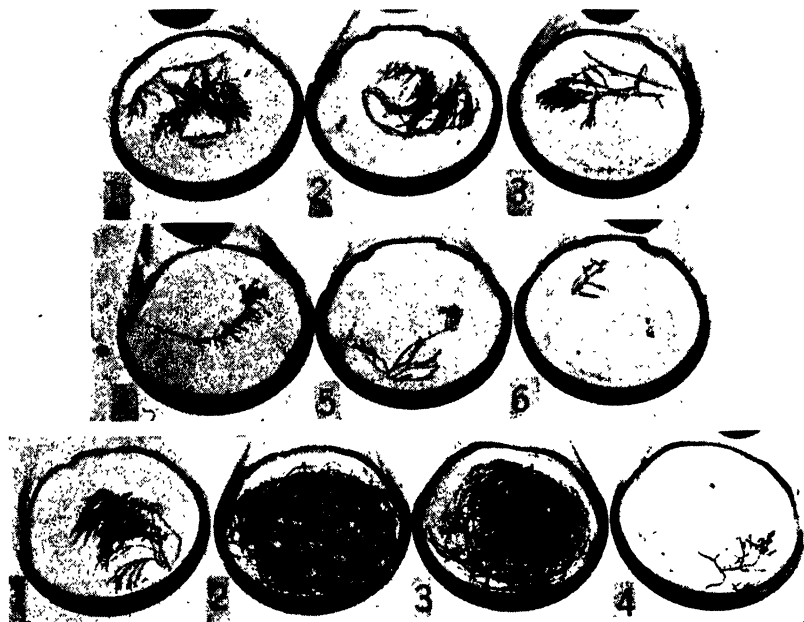
FIG. 11.—Relation between dry weights of individual roots and amount of vitamin B<sub>1</sub> or vitamin thiazole supplied. Grown in WHITE's solution with yeast and cane sugar replaced by light brown commercial cane sugar. Logarithms of amount of supplement plotted against dry weight in mg.

creased growth occurred in the solutions containing up to 100 gamma.

Some solutions were included in passage 20, in which the sugar medium was supplemented with 10 gamma each of thiazole and bromopyrimidine. The roots grown in these solutions were indistinguishable from those grown with 10 gamma of vitamin B<sub>1</sub>; the average dry weight of four roots was 69.4 mg., which is within the

range obtained with the higher concentrations of vitamin B<sub>1</sub> in the same medium (table 11). The similarity of the roots in the medium containing both thiazole and bromopyrimidine and those in the solution containing vitamin B<sub>1</sub> is shown in figure 13.

When various dilutions of mixtures of thiazole and pyrimidine were used, however, growth was not so good in the more dilute solu-



FIGS. 12, 13.—Fig. 12 (above), effect of various amounts of vitamin thiazole. Roots grown in WHITE'S solution with yeast and cane sugar replaced by light brown commercial sugar plus: 1, 100 gamma thiazole; 2, 10 gamma; 3, 1.0; 4, 0.1; 5, 0.01; 6, none. Fig. 13 (below), effect of vitamin B<sub>1</sub> and intermediates on growth of roots grown in WHITE'S solution with yeast and cane sugar replaced by light brown commercial sugar plus: 1, 10 gamma vitamin thiazole; 2, same plus 10 gamma vitamin pyrimidine; 3, 10 gamma vitamin B<sub>1</sub>; 4, 10 gamma vitamin pyrimidine.

tions containing the intermediates as in those containing the vitamin.

In passage 21, begun June 9, 1937, a series of cultures were made in various concentrations of vitamin B<sub>1</sub>, of thiazole, of pyrimidine, and of a mixture of thiazole and pyrimidine. The basic medium was WHITE'S mineral solution with 2% light brown sugar treated with



charcoal. Inoculum came from roots grown in WHITE's solution with Eastman's maltose replacing sucrose. The pieces of inoculum were rinsed in sterile mineral solution before they were placed in the culture flasks in order to remove the last traces of the medium in which they had grown.

On July 9, after 1 month of growth, roots in the mixture of thiazole and pyrimidine at the higher concentrations (10, 1.0, and

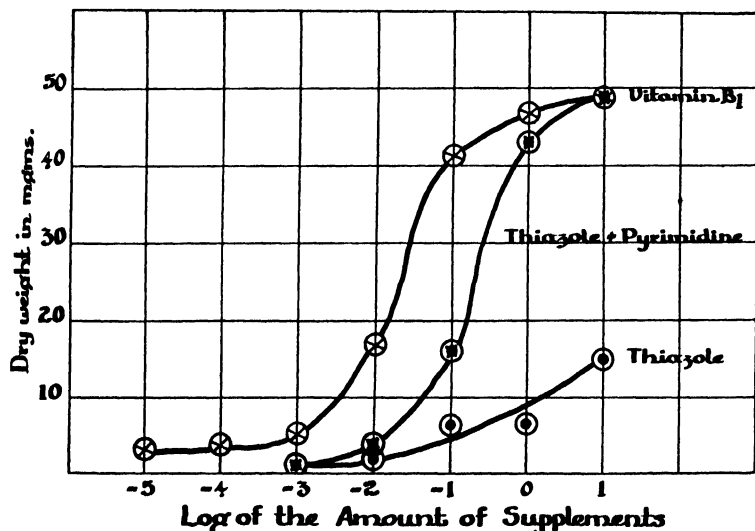


FIG. 14.—Effect of vitamin B<sub>1</sub>, thiazole, and mixtures of thiazole and pyrimidine. Roots grown in WHITE's solution with yeast and cane sugar replaced by light brown sugar treated with charcoal. Logarithm of amount of supplements plotted against dry weight of roots in mg.

0.1 gamma per flask) resembled the roots in similar solutions containing the same concentrations of vitamin B<sub>1</sub>; but a very decided difference was observed in the lower concentrations. This difference was obvious when the experiment was terminated in October, as shown by the curves in figure 14 and the data on dry weights in table 12.

In passage 21, the effect of 0.0001 gamma of vitamin B<sub>1</sub> was evident and the maximum growth was secured in the solutions containing 0.1 gamma or more. The charcoal evidently removed some beneficial material from the sugar, as the maximum growth in the

solutions containing the treated sugar (about 48 mg.) was not so great as in those which contained the untreated sugar (about 75 mg.). The results with different amounts of thiazole were similar to those secured with the untreated sugar in passage 20, except for those obtained with 0.0001 gamma of thiazole (table 12). No explanation other than experimental error can be offered for the aberrant results with this amount of thiazole.

TABLE 12

AVERAGE DRY WEIGHTS OF ROOTS FROM PASSAGE 21 IN WHITE'S MINERAL SOLUTION AND 2% LIGHT BROWN SUGAR TREATED WITH CHARCOAL AND SUPPLEMENTED WITH VARIOUS AMOUNTS OF VITAMIN B<sub>1</sub> OR ITS INTERMEDIATES

AMOUNT SUPPLIED IN GAMMA PER FLASK	VITAMIN B <sub>1</sub>		THIAZOLE		THIAZOLE AND PYRIMIDINE		PYRIMIDINE	
	No. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	No. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	No. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	No. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)
10.0.....	5	48.8	4	14.8	5	48.2	3	1.1
1.0.....	4	46.9	5	6.7	3	43.2	5	1.1
0.1.....	5	41.7	4	6.4	5	16.0	5	1.1
0.01.....	4	17.5	4	1.5	4	2.9	3	0.9
0.001.....	5	5.1	5	0.8	5	1.0	4	1.0
0.0001.....	5	3.8	4	3.2	4	0.3	4	1.0
None.....	3	0.7	3	0.7	3	0.7	3	0.7

No growth occurred with any of the amounts of pyrimidine used, except for two roots. One root in the medium composed of WHITE's mineral solution, 2% light brown sugar treated with charcoal, and 0.01 gamma of pyrimidine per flask grew as well as the roots with the same amount of vitamin B<sub>1</sub> in the same medium. The root in the solution supplemented with 0.01 gamma of pyrimidine weighed 17.4 mg. and the average for the roots in the medium with the same concentration of vitamin B<sub>1</sub> was 17.5 mg. The other root, grown in the same medium with 0.001 gamma of pyrimidine in the flask, weighed 4.5 mg.; and the average for roots in the solution with vitamin B<sub>1</sub> at the same concentration was 5.1 mg. When sketches of the inocula were examined, no difference was found which could explain the re-

sults, although this did not exclude the possibility that the particular pieces used might have differed in other respects than in appearance.

It was suggested that certain pieces may have been in a state of active growth, and having once started well, were able to grow in an unfavorable medium. To test this the following experiment was performed. In passage 22, roots from WHITE's solution with Eastman's maltose replacing sucrose were transferred to a medium composed of WHITE's mineral solution and 2% light brown sugar treated with charcoal, supplemented by vitamin B<sub>1</sub> at 0.0001 or 0.000001 gamma per flask. These concentrations of the vitamin had been found to produce some growth (table 11). The roots were allowed to grow for 2 weeks; then pyrimidine was added at the concentrations which had given growth in passage 21, namely, 0.01 or 0.001 gamma per flask. If our assumptions were correct, growth should have continued after the pyrimidine was added. The pyrimidine did not influence growth. No explanation other than experimental error can be offered for the growth of the two roots in the pyrimidine cultures.

The experiments described were performed with an obviously impure material, light brown sugar. What is the effect of dilution of the vitamin in a solution prepared with pure chemicals? The experiments with pure chemicals were not so elaborate as those with the light brown sugar medium. They showed, however, that amounts of vitamin B<sub>1</sub> as low as 0.0001 gamma affected the growth of the roots favorably.

In passage 15, begun November 24, 1936, besides subculturing roots in WHITE's solution with 10 gamma of B<sub>1</sub> replacing the yeast, four other concentrations were tested: 50, 1, 0.01, and 0.001 gamma per flask containing 50 ml. of medium. On December 6, after 10 days in the culture solutions, it was noted that roots were growing at each concentration (table 13).

In a second experiment (passage 16) roots were grown in WHITE's solution with yeast replaced by 10, 1, 0.01, 0.001, or 0.0001 gamma of vitamin B<sub>1</sub> per flask. A small but definite effect of 0.0001 gamma of the vitamin was noted by the appearance of the roots and in the dry weights.

The maximum growth in WHITE's solution with vitamin B<sub>1</sub> in-

stead of yeast did not equal that secured in the solutions in which the light brown sugar was used. In figure 15 the effect is shown of different amounts of vitamin B<sub>1</sub> in WHITE's solution lacking yeast, and in WHITE's solution with cane sugar and yeast replaced by vitamin B<sub>1</sub> and light brown sugar, or light brown sugar treated with charcoal. From these curves it is clear that something other than the amount of vitamin B<sub>1</sub> limited growth in WHITE's solution (lacking yeast) at the higher concentrations of the vitamin. We are inclined to believe that the limiting factor in WHITE's solution (with

TABLE 13

DRY WEIGHTS OF ROOTS FROM PASSAGE 15 GROWN IN WHITE'S SOLUTION AND IN SAME WITH YEAST EXTRACT REPLACED BY VARIOUS CONCENTRATIONS OF VITAMIN B<sub>1</sub>

ADDITIONS TO WHITE'S SOLUTION WITHOUT YEAST	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
50 gamma vitamin B <sub>1</sub> per flask . . . .	9	8.2	1.7-12.0
1 gamma vitamin B <sub>1</sub> per flask . . . .	8	8.8	7.5-10.1
0.01 gamma vitamin B <sub>1</sub> per flask . . .	6	3.5	1.1-6.9
0.001 gamma vitamin B <sub>1</sub> per flask . .	8	7.2	1.7-10.2
Yeast extract 100 p.p.m. . . . .	5	7.8	3.3-10.4

vitamin B<sub>1</sub> instead of yeast) was probably the nature or amounts of mineral salts.

Although we have noted an effect of 0.000001 gamma of vitamin B<sub>1</sub> in the brown sugar medium, and of 0.0001 gamma in WHITE's solution lacking yeast, this does not imply that continued growth would occur in media containing so small an amount of the vitamin. In fact, in these experiments tomato roots ceased to grow when carried through successive passages in solutions containing 0.001 gamma or less of vitamin B<sub>1</sub>. We have not determined the minimum amount of the vitamin necessary for unlimited growth of excised tomato roots. It is probably in the vicinity of 0.1 gamma per flask under the conditions of the experiments. It would be influenced by the duration of the individual passages, which would determine in part the total amount of vitamin available to a particular excised root in a given period of time.

It may be said, therefore, that while growth occurred with very low concentrations of vitamin B<sub>1</sub>, solutions containing less than 0.001 gamma were not adequate to maintain the roots through successive passages. A concentration of 10 gamma of vitamin B<sub>1</sub> per flask, or 0.2 gamma per ml., was sufficient to maintain growth through thirteen passages extending over a period of 14 months.

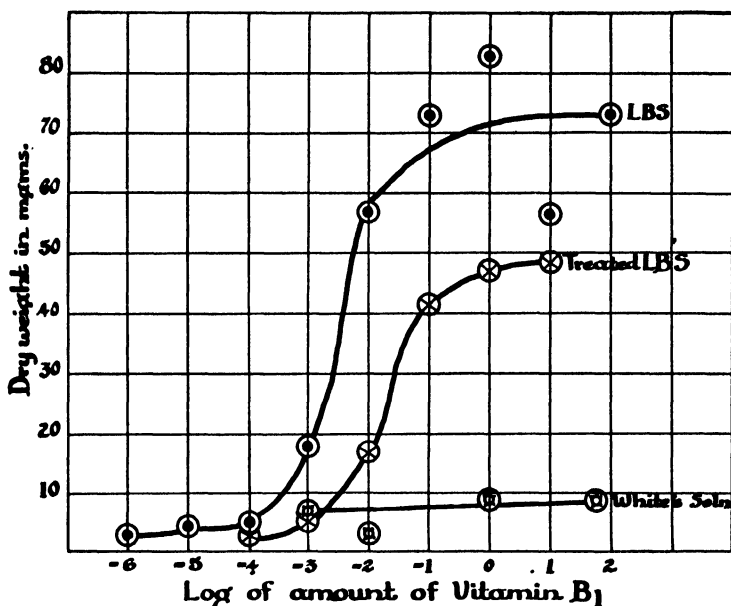


FIG. 15.—Effect of vitamin B<sub>1</sub> in different solutions. Logarithm of amount of vitamin B<sub>1</sub> plotted against dry weight of roots in mg. Below, WHITE's solution with yeast replaced by vitamin B<sub>1</sub>. Above, WHITE's solution with yeast and cane sugar replaced by vitamin B<sub>1</sub> and light brown sugar. Middle, WHITE's solution with yeast and cane sugar replaced by vitamin B<sub>1</sub> and light brown sugar treated with charcoal.

**CARBON SOURCES FOR EXCISED TOMATO ROOTS.**—WHITE's solution contains sucrose as the source of carbon. Can tomato roots use carbon sources other than sucrose, and what is their relative value? We have investigated dextrose, levulose, maltose, mannose, cellobiose, inositol, pyruvic acid, and several samples of commercial cane sugar.<sup>4</sup> Dextrose and maltose proved satisfactory substitutes for cane sugar. Some growth was secured with levulose and cellobiose,

<sup>4</sup> All carbon sources were used at 2% concentration unless otherwise stated.

but none with the samples of mannose, inositol, or pyruvic acid used.

A. DEXTROSE AND MALTOSE.—In passage 11, seven pieces of inoculum were transferred to WHITE's solution containing Merck maltose, purified, no. 30504, instead of cane sugar, and seven to WHITE's medium with dextrose, Pfanstiehl's c.p. d-glucose no. 380, replacing the sucrose. The roots grew well in the solutions containing maltose or dextrose, but showed a characteristic type of growth different from that of those grown in WHITE's solution with sucrose. Excised roots in the sucrose cultures had short branches, many tips were swollen, and growth did not continue for much more than 1 month. In WHITE's solution with dextrose replacing sucrose the increase in length of branches was rapid, resulting in longer branches; the roots were more slender and were translucent rather than yellowish white. When examined with a hand lens, the roots in the dextrose solution showed noticeably fewer root hairs than did those in WHITE's solution with sucrose. A swollen condition of the tips was sometimes noticeable after  $1\frac{1}{2}$  to 2 months in the dextrose cultures, but it was not so pronounced as in the WHITE's solution with sucrose. The type of growth in the maltose cultures was different from that in either the dextrose or sucrose solutions. The branches were long, slender, and very white; root hairs were more numerous than in the dextrose cultures. Swollen tips were rare in the maltose cultures; and growth continued for more than 2 months. Roots grown with these three sugars are shown in figure 16. It was not easy to determine when growth in the maltose solutions actually stopped, for the roots appeared white and the tips were normal when examined after 5 months of growth.

Subcultures of the roots in dextrose and in maltose were made to the same solutions for a number of successive passages (table 14). The same types of growth were obtained throughout the experiments with maltose and dextrose.

From the experiments it appeared that excised tomato roots were able to utilize dextrose, although WHITE (30) has reported otherwise. We have carried roots through six successive passages (table 14) in WHITE's solution with sucrose replaced by dextrose. In fact, so far as dry weights are concerned, the dextrose was at least as

satisfactory as cane sugar. Two samples of dextrose were used, Pfanstiehl's c.p. d-glucose and cerelose (in one passage only). The temperature of sterilization ( $110^{\circ}$  or  $120^{\circ}$  C.) of the dextrose had little effect upon growth of the roots (passage 14, table 14). Growth occurred also in dextrose solutions which were filtered sterile. It

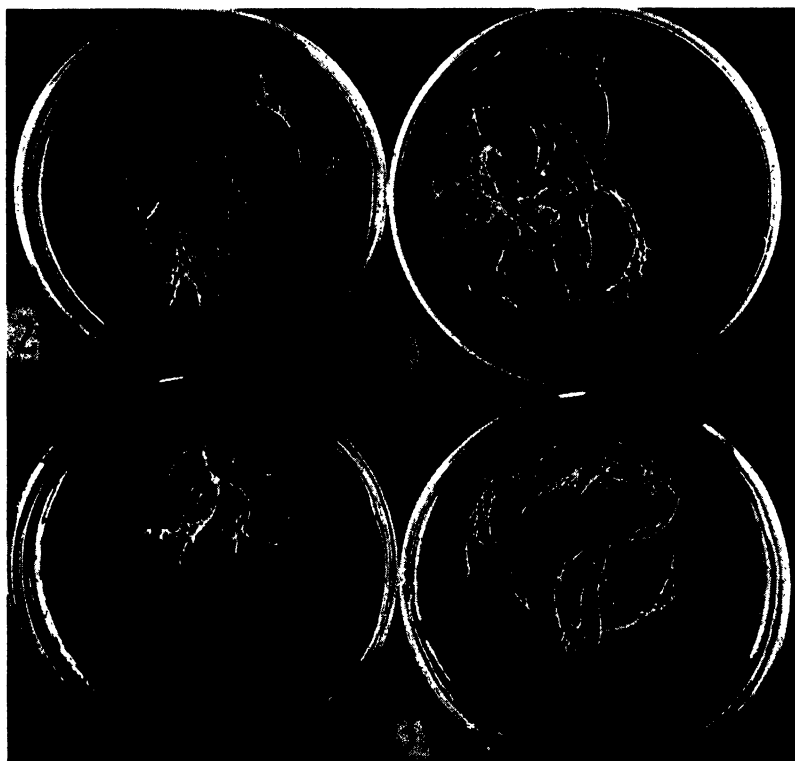


FIG. 16.—Various carbohydrates and growth of tomato roots: 1, WHITE's solution (cane sugar); 2, cane sugar replaced by dextrose; 3, by maltose; 4, cane sugar and yeast replaced by maltose.

was found further that subcultures of the clone of excised tomato roots used by WHITE<sup>5</sup> grew in a dextrose solution. These results have been discussed elsewhere (20).

We are not certain whether the difference between the character of the growth noted in media containing dextrose and that in those

<sup>5</sup> These subcultures were furnished through the courtesy of Dr. P. R. WHITE.

containing cane sugar was associated with the carbohydrate, or with the traces of contaminants present in these purified sugars. WHITE's solution can be improved by the addition of mineral supplements (see discussion of mineral nutrients later), and all of the carbohydrates used contain traces of many elements. For example, al-

TABLE 14

DRY WEIGHTS OF ROOTS GROWN THROUGH SUCCESSIVE PASSAGES IN (A) WHITE'S SOLUTION WITH SUCROSE REPLACED BY DEXTROSE; (B) SAME WITH SUCROSE REPLACED BY MERCK'S MALTOSE NO. 30504; (C) WHITE'S SOLUTION. IN SOLUTION A THE DEXTROSE WAS PFANSTIEHL'S DEXTROSE NO. 380; IN A', CEREOSE

PASSAGE	CULTURE SOLUTION	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
11.....	{ A	5	25.7	17.5-30.0
	{ B	5	62.4	54.7-72.4
	{ C	7	14.8	7.0-19.5
12.....	{ A	4	10.5	6.3-12.6
	{ B	5	25.5	19.4-26.7
	{ C	6	22.3	17.0-29.2
13.....	{ A	4	11.6	9.7-14.5
	{ B	4	14.4	5.8-20.8
	{ C	6	8.5	4.5-11.0
14*.....	{ A-110° C.	7	12.2	1.7-21.4
	{ A-120° C.	7	13.1	4.5-24.6
	{ B-110° C.	8	10.4	0.2-33.8
	{ B-120° C.	5	12.1	2.4-18.7
	{ C-110° C.	9	6.7	4.2-12.1
	{ C-120° C.	6	8.0	6.6-10.5
15.....	{ A	7	25.4	18.1-29.6
	{ C	5	7.8	3.3-10.4
16.....	{ A	9	4.5	1.1-11.4
	{ A'	7	7.1	1.8-10.4
	{ C	28	5.4	0.9-10.4

\* Some solutions sterilized at 110° C. for 30 minutes and others at 120° C. for 20 minutes.

though our sample of cerelose had only 0.004% ash, it contained traces of twenty elements, as shown by spectrographic analysis. Qualitative spectrographic analyses were made by arcing the ash of cerelose (dextrose) directly on graphite electrodes by A. P. VANSELOW through the courtesy of Dr. W. P. KELLEY of the University



of California. Elements present in large amounts were Ca and Na; in definite traces, Ag, Al, Ba, Cr, Cu, Fe, K, Mg, Ni, Pb, and Sr; and elements just detectable, B, Mn, Pt, Si, Sn, Ti, and Zn. Although an analysis of the ash of other carbon sources was not made, all the samples used contained minerals in varying amounts, as shown in table 15.

In these experiments the effect of any carbon source, even its apparent availability, was found to depend to a considerable extent on the particular sample used. This was true even with the purified

TABLE 15  
ASH CONTENTS OF SUGARS USED AS CARBON SOURCES

CARBOHYDRATE	SAMPLE	PERCENTAGE ASH
Dextrose.....	{Pfanstiehl no. 380	0.008
	{Cerelese	0.004
Maltose.....	{Pfanstiehl no. 602	0.358
	{Merck no. 30516	0.092
	{Eastman	0.138
Sucrose.....	{Pfanstiehl no. 409	>0.00001
	{Light brown	1.909
	{Dark brown	3.380

samples. These statements may be illustrated by the results with maltose. Merck's purified maltose no. 30504 proved entirely satisfactory as a source of carbon for excised tomato roots. In four successive passages (table 14) the growth in solutions containing this maltose was superior to that in the solutions containing cane sugar or dextrose. However, Pfanstiehl's c.p. maltose no. 602 was decidedly injurious; no growth was obtained in several trials with this sugar replacing sucrose in WHITE's solution. A sample of Eastman's maltose was almost as satisfactory as Merck no. 30504. Growth with Merck purified maltose no. 30516 was poor; with Merck purified maltose no. 22068 the growth was better, but not so good as with the Eastman maltose. Depending upon the sample used, it would be possible to conclude that maltose was not available to tomato roots, that maltose was better than cane sugar, or that maltose was poorer than cane sugar as a source of carbon.

It is noteworthy, however, that in each instance where growth occurred in the maltose solutions the roots had the same general appearance, irrespective of the sample of maltose used. They were white in color, had normal appearing tips on their branches, and produced relatively long branches, not short and stubby ones as were frequent in the sucrose solutions.

In addition to the experiments in which different samples of maltose were used to replace sucrose in WHITE's solution, a number of the same samples of maltose, which had been found to be satisfactory carbon sources, were used to replace both sucrose and the yeast extract in WHITE's medium. In general the samples which had proved most satisfactory as carbon sources were best when used in place of both sugar and yeast.

B. LEVULOSE AND CELLOBIOSE.—In passage 16, subcultures were made in WHITE's solution with cane sugar replaced by levulose or by cellobiose. Both compounds came from the Eastman Kodak Co. The amount of growth in the levulose solutions was almost as great as in WHITE's solution, but was considerably less in the cellobiose solutions. No subcultures were made in solutions containing these two carbon sources, and only the one sample of each was tested. The type of growth in the two media was similar to that obtained with dextrose: long, slender, translucent roots, with few root hairs.

C. MANNOSE, PYRUVIC ACID, AND INOSITOL.—In passage 19, mannose and pyruvic acid were tested as possible carbon sources for excised tomato roots. The mannose was Pfanstiehl's sample no. 587, and the pyruvic acid was an Eastman product. Mannose was used at 2% concentration; and pyruvic acid, adjusted to pH 5.0, was used at two concentrations, 0.5 and 0.1%. No growth was obtained with either of these carbon sources. Other samples of these compounds were not at hand, and no final conclusion as to availability of mannose and pyruvic acid can be drawn.

In passage 22, a sample of inositol, an Eastman product, was used in place of sucrose in solution W plus vitamin B<sub>1</sub>. No growth was obtained with this sample of inositol.

D. COMMERCIAL CANE SUGARS.—In addition to the pure (Pfanstiehl c.p.) cane sugar used in WHITE's solution, four samples of commercial cane sugar have been used: a dark brown sugar, two

light brown sugars, and a white granulated sugar. The dark brown sugar was injurious. No growth occurred in WHITE's solution with the pure cane sugar replaced by the dark brown sugar. The white granulated sugar also appeared to be somewhat injurious. Poorer growth was secured in solution Z containing this sugar and yeast than in the same solution containing the "chemically pure" sugar. However, the medium containing the light brown sugars proved

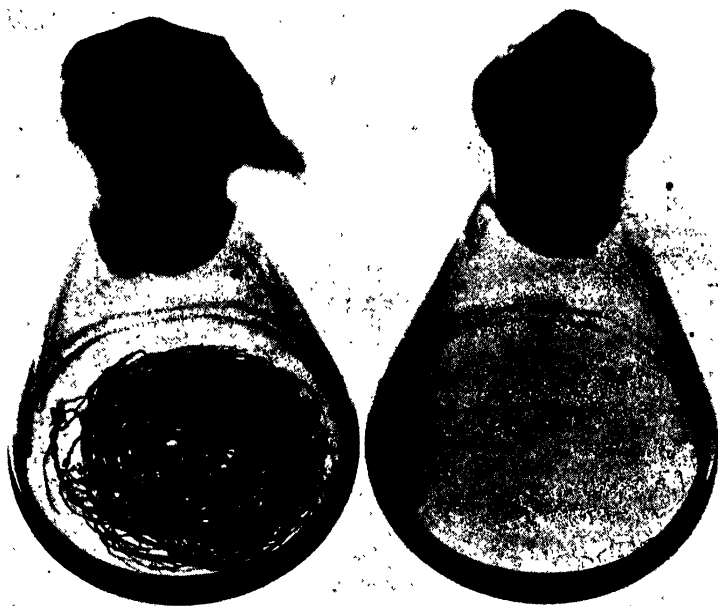


FIG. 17.—Effect of vitamin B<sub>1</sub>. WHITE's solution with yeast and cane sugar replaced by light brown sugar: left, plus 10 gamma vitamin B<sub>1</sub> (dry wt. 80.0 mg.); right, no vitamin B<sub>1</sub> (dry wt. 1.0 mg.).

superior to any medium we have used. In fact, so excellent was the growth with this impure sugar that it was used in a number of our experiments with vitamin B<sub>1</sub> or intermediates (fig. 17).

The only other medium which gave growth comparable with that obtained in the light brown sugar medium was one composed of WHITE's minerals, "chemically pure" sucrose, and a water extract of malt flour. The malt flour cultures in which fresh malt flour ex-

tract was added four times at intervals of 2 weeks gave growth within the range of that obtained with light brown sugar.

The largest root (dry weight 91.3 mg.) obtained in our experiments was grown in a medium composed of WHITE's mineral solution, 2% light brown sugar, and 10 gamma of vitamin B<sub>1</sub> per flask. The root was grown at room temperature and remained in the flask from March 5, 1937 until October 20, 1937. It is not known whether the root was still growing when the experiments were terminated, for measurements at intervals were not possible; however, many of the branches were white at the tips and appeared normal at the end of the experiments.

We are not prepared to explain the highly beneficial effect of the light brown sugar. It was evidently not because of its content of vitamin B<sub>1</sub> nor of thiazole. Very little growth was secured in solutions containing this sugar unless vitamin B<sub>1</sub> or thiazole was also present. The ash of the sugar used in amounts equivalent to that in a 2% solution of the sugar proved toxic. It is possible, however, that smaller amounts of the ash might be beneficial. Part of the beneficial material was removed by treatment with Merck medicinal charcoal or with Fuller's earth. The ash content (1.9%) of this sugar was high and its beneficial action may be associated with the minerals it contains. Our experiments, however, have not eliminated the possibility that organic material other than sucrose, vitamin B<sub>1</sub>, or thiazole may be concerned.

**MINERAL NUTRIENTS.**—The mineral salts in WHITE's solution are supplied by a known mixture of the composition given earlier. In addition there is an unknown mixture of minerals added in the yeast extract. The concentration of salts from the known mixture in WHITE's solution was about 375 p.p.m. From the ash of a water extract of yeast it is estimated that about 5 p.p.m. of mineral material was added with the yeast extract used in WHITE's solution. Are the minerals in the yeast extract of significance in determining growth in WHITE's solution? Is the known mixture of salts in WHITE's solution adequate for the best growth of excised tomato roots? Can some other mixture be substituted for it?

In our experiments the ash elements added with the yeast appeared to be of some importance. The growth in WHITE's solution

in which the yeast was replaced by vitamin B<sub>1</sub> and yeast ash was superior to that in the same solution in which the yeast was replaced by vitamin B<sub>1</sub> alone. In fact the vitamin and yeast ash together gave results somewhat better than the yeast.

Some attempt was made to determine what specific mineral substances were effective in the yeast ash. Since the yeast was added to a solution which already contained the common essential mineral elements, our attention was directed toward the rarer elements. The effects of boron, zinc, and a modification of Hoagland's A to Z mix-

TABLE 16

DRY WEIGHTS OF ROOTS FROM PASSAGE 18 GROWN IN WHITE'S SOLUTION AND THE SAME WITH YEAST REPLACED BY VITAMIN B<sub>1</sub> ALONE OR BY VITAMIN B<sub>1</sub> AND MINERAL SUPPLEMENTS

SOLUTION	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
White's solution with vitamin replacing yeast.....	9	9.2	2.6-11.2
Solution W plus vitamin B <sub>1</sub> .....	10	12.2	0.4-20.3
Solution Z plus vitamin B <sub>1</sub> .....	10	9.3	0.3-39.9
White's solution.....	14	14.1	7.7-23.4

ture were tested (fig. 20). When the yeast in WHITE's solution was replaced by vitamin B<sub>1</sub>, 0.1 p.p.m. of B (as boric acid), and 0.1 p.p.m. of Zn (as zinc sulphate), the growth was almost as good as in WHITE's solution and superior to that in WHITE's solution with yeast replaced by vitamin B<sub>1</sub> alone (table 16). It would seem, therefore, that a part of the beneficial action of the yeast extract is related to its boron and zinc content.

Results with the modification of Hoagland's A to Z mixture were irregular. In some experiments a decidedly favorable effect was observed, in others the mixture appeared to be somewhat injurious. It is possible that a dilution of the mixture would be preferable.

While the mixture of salts in solution W was superior to the known mixture in WHITE's solution, it is probable that a further improvement could be made. This is suggested by the fact that growth in WHITE's solution was somewhat superior to that in the same

medium with yeast replaced by vitamin B<sub>1</sub>, boron, and zinc and because some samples of filter paper or of filter paper ash improved growth when added to WHITE's solution in which yeast was replaced by vitamin B<sub>1</sub>, boron, and zinc.

In passage 6, WHITE's solution was supplemented by adding  $\frac{1}{2}$  sheet of a sample of A. H. Thomas qualitative filter paper to each flask. ROBBINS and WHITE (16) had found this filter paper beneficial to excised corn roots. It was also effective as a supplement to WHITE's solution for tomato roots. Excised roots of tomato grown

TABLE 17

DRY WEIGHTS OF ROOTS GROWN IN WHITE'S SOLUTION  
AND IN SAME SOLUTION SUPPLEMENTED BY TWO  
SAMPLES OF FILTER PAPER

PASSAGE	ADDITIONS TO WHITE'S SOLUTION	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
6.....	{None	6	14.6	10.0-17.5
	{Filter paper	9	46.7	32.0-71.0
18.....	{None	14	14.1	7.7-23.4
	{Filter paper	6	20.3	11.2-32.2

in WHITE's solution containing filter paper had much longer branches and were whiter in color than those in WHITE's solution (fig. 18). In passage 18 another sample of A. H. Thomas qualitative filter paper was used. Again beneficial results were obtained; but they were not so marked as were those in the earlier experiment (table 17). A third sample of filter paper completely inhibited growth.

If filter paper added something to WHITE's solution which made it more suitable for growth, was it mineral in character? To test this, filter paper ash was used as a supplement. The results varied with the sample of filter paper used and the treatment of the ash. The ash of some samples was highly beneficial, especially when treated with hydrochloric acid; that of others was injurious. In passage 15 filter paper ash was used in WHITE's solution with vitamin B<sub>1</sub> replacing the yeast. Ash of  $\frac{1}{2}$  sheet of filter paper per flask was used, but practically no growth was obtained (table 18). In a later experiment, passage 22, the same sample of filter paper ash was

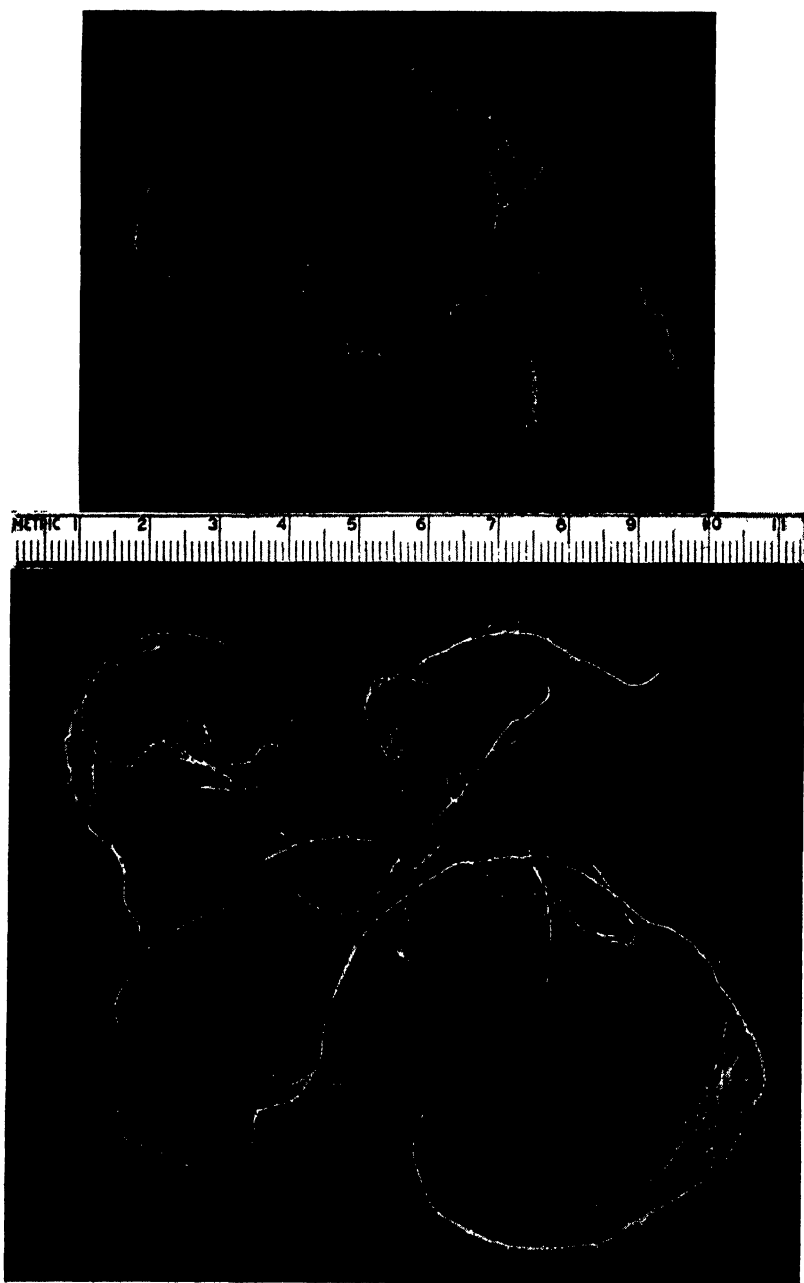


FIG. 18.—Effect of filter paper (passage 6): above, excised root in WHITE's solution; below, same plus  $\frac{1}{2}$  sheet filter paper.

treated with hydrochloric acid, the acid boiled off, and the residue used in solution W plus vitamin B<sub>1</sub>. In this experiment the filter paper ash proved highly beneficial (table 18). Two out of three roots weighed were much superior to any of those in solution W with vitamin B<sub>1</sub> alone in this and other experiments. The best roots from the two sets are shown in figure 19.

TABLE 18

DRY WEIGHTS OF ROOTS SHOWING EFFECTS OF ASH OF FILTER PAPER  
AND OF SAME ASH TREATED WITH HYDROCHLORIC ACID

PASSAGE	ADDITIONS TO WHITE'S SOLUTION WITH VITAMIN B <sub>1</sub> REPLACING YEAST	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
15.....	{None Filter paper ash (untreated)	7	7.1	2.7-17.1
		9	0.5	0.3-0.9
22.....	{Boron and zinc 0.1 p.p.m. Boron and zinc plus filter paper ash treated with HCl	6	7.5	5.2-8.2
		3	23.6	3.0-45.5

Are the concentrations and actions of salts used in the known mixture in WHITE'S solution more satisfactory than other solutions? We have used two other basic mineral solutions:

Solution C (16), with the following composition:

Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.25 gm.
MgSO <sub>4</sub> .....	0.05
KH <sub>2</sub> PO <sub>4</sub> .....	0.05
FeCl <sub>3</sub> .....	0.005
H <sub>2</sub> O.....	5000.00 ml.

A modified Pfeffer's solution (10):

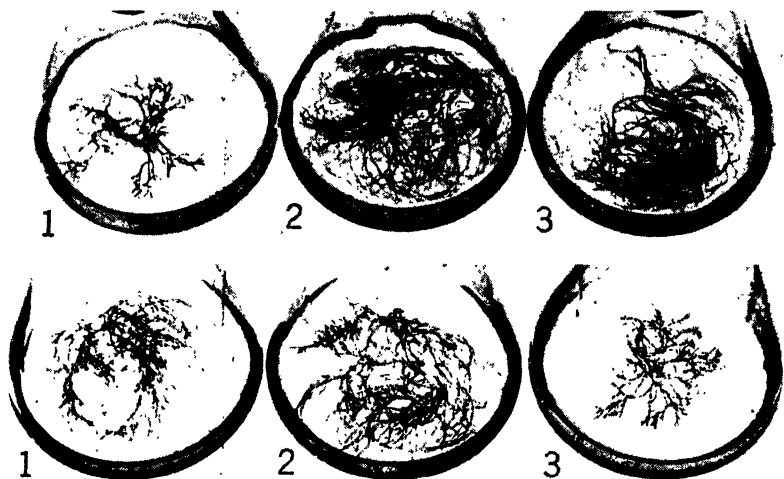
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	2.0 gm.
KH <sub>2</sub> PO <sub>4</sub> .....	0.5
KNO <sub>3</sub> .....	0.5
KCl.....	0.25
MgSO <sub>4</sub> .....	0.5
FeCl <sub>3</sub> .....	0.005
H <sub>2</sub> O.....	6000.00 ml.

No appreciable growth was secured when either of these mineral solutions was used with the same concentration of yeast and cane sugar as is present in WHITE'S solution. When the FeCl<sub>3</sub> in Pfeffer's



solution was replaced by 2.5 p.p.m.  $\text{Fe}_2(\text{SO}_4)_3$ , however, growth occurred although not to so great an extent as in WHITE's solution.

The modified Pfeffer's solution with sucrose and water extract of yeast and 2.5 p.p.m.  $\text{Fe}_2(\text{SO}_4)_3$  was not so satisfactory as WHITE's solution. However, the modified Pfeffer's solution with 2.5 p.p.m. of  $\text{Fe}_2(\text{SO}_4)_3$  plus 0.1 p.p.m. of B and 0.1 p.p.m. of Zn proved superior to solution W when each solution was supplemented with vitamin  $\text{B}_1$ .



FIGS. 19, 20.—Fig. 19 (above), effect of filter paper ash (passage 22). Roots grown in: 1, solution W plus vitamin  $\text{B}_1$ ; 2, same plus filter paper ash treated with  $\text{HCl}$ ; 3, WHITE's solution with Eastman's maltose instead of cane sugar. Fig. 20 (below), effect of mineral supplements. Roots grown in WHITE's solution with yeast replaced by vitamin  $\text{B}_1$ : 1, plus 0.1 p.p.m. boron and zinc; 2, plus A to Z mixture; 3, no addition.

In passage 22, excised tomato roots were grown in: (a) WHITE's mineral solution; (b) modified Pfeffer's solution; (c) modified Pfeffer's solution with 2.5 p.p.m. of  $\text{Fe}_2(\text{SO}_4)_3$ ; (d) modified Pfeffer's solution with 3.2 p.p.m.  $\text{FeCl}_3$ . To each solution 2% cane sugar, 0.1 p.p.m. B, 0.1 p.p.m. Zn, and vitamin  $\text{B}_1$  were added. In passage 23, subcultures of roots grown in these four solutions were made to the same media. Results of this experiment are summarized in table 19.

The Pfeffer's solution containing 0.1 p.p.m. of B and Zn, 2% cane sugar, and vitamin  $\text{B}_1$  was not satisfactory (table 19), but when the

0.8 p.p.m. of  $\text{FeCl}_3$  in this solution was replaced by 2.5 p.p.m. of  $\text{Fe}_2(\text{SO}_4)_3$  it proved superior to WHITE's mineral solution containing the B, Zn, cane sugar, and vitamin  $\text{B}_1$ . When the  $\text{Fe}_2(\text{SO}_4)_3$  in the Pfeffer's solution was replaced by  $\text{FeCl}_3$ , containing an equivalent amount of Fe, the results were about the same as those secured with WHITE's mineral solution, but decidedly inferior to those with the Pfeffer's solution containing 2.5 p.p.m.  $\text{Fe}_2(\text{SO}_4)_3$ .

TABLE 19

DRY WEIGHTS OF ROOTS THROUGH TWO PASSAGES IN MEDIUM COMPOSED OF MINERAL SOLUTION PLUS BORON AND ZINC 0.1 P.P.M., 2% SUCROSE AND VITAMIN  $\text{B}_1$ : (A) WHITE'S MINERAL SOLUTION; (B) MODIFIED PFEFFER'S SOLUTION; (C) SAME WITH 2.5 P.P.M.  $\text{Fe}_2(\text{SO}_4)_3$  REPLACING THE  $\text{FeCl}_3$ ; (D) SAME WITH 3.2 P.P.M.  $\text{FeCl}_3$  REPLACING THE 0.8 P.P.M.  $\text{FeCl}_3$

PASSAGE	MINERAL SOLUTION USED	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
22.....	{ A	5	4.9	0.6-9.7
	{ B	4	2.9	2.5-3.2
	{ C	4	20.1	16.6-22.3
	{ D	3	6.2	4.0-8.5
23.....	{ A	7	5.6	0.4-9.6
	{ B	4	1.6	0.9-2.1
	{ C	4	12.1	10.5-15.5
	{ D	4	4.2	3.7-5.5

Why should the  $\text{Fe}_2(\text{SO}_4)_3$  be superior to the  $\text{FeCl}_3$ ? Possibly not because of the Fe content but more probably because of a greater amount of contaminants in the  $\text{Fe}_2(\text{SO}_4)_3$ , perhaps manganese. Analyses of the  $\text{FeCl}_3$  by the periodate method showed the presence of 0.0013% Mn. The  $\text{Fe}_2(\text{SO}_4)_3$  contained approximately forty times this amount of Mn.

SOURCES OF NITROGEN.—WHITE's solution contains  $\text{Ca}(\text{NO}_3)_2$  and  $\text{KNO}_3$ . In addition, amino acids and other types of nitrogenous compounds are supplied in the extract of yeast. Is the organic nitrogen in WHITE's solution important as a source of nitrogen? Can excised tomato roots utilize nitrates as a source of nitrogen?

In our experiments excised tomato roots appeared to be capable of utilizing nitrates; in fact, they required no other source of nitro-

gen. This conclusion followed because the roots grew through fourteen passages over a period of more than 14 months in a solution of mineral salts, pure cane sugar, and synthetic vitamin B<sub>1</sub>. When the nitrates were omitted from this solution no growth occurred.

For the first 4 months WHITE's solution with the yeast replaced by 10 gamma of vitamin B<sub>1</sub> per flask was used and for the last 10 months solution W plus cane sugar and vitamin B<sub>1</sub>. The nitrogen furnished by the vitamin in these solutions was 0.03 gamma N per ml., or 1.5 gamma per flask. The nitrates in WHITE's solution or in solution W furnished 28 gamma of N per ml., or 1400 gamma per flask.

To test the possibility that vitamin B<sub>1</sub>, even at this low concentration, might furnish nitrogen for growth of the roots, solutions without nitrates were tried. In passage 22, root fragments were transferred to WHITE's solution with vitamin B<sub>1</sub> replacing the yeast, and to the same solution without nitrates. The Ca(NO<sub>3</sub>)<sub>2</sub> and KNO<sub>3</sub> were replaced by CaCl<sub>2</sub> and KCl respectively. No growth was visible in the solution without nitrates.

In this same passage, ammonia was used in place of nitrates. Ammonium chloride, equivalent in nitrogen to that in the nitrates, was used in WHITE's solution without nitrates. No growth was obtained. In passage 24, both ammonium sulphate and ammonium chloride were used as substitutes for nitrates in WHITE's mineral solution; they were used at concentrations such that the nitrogen was one-tenth that in WHITE's solution. No growth occurred. As an additional check, ammonium chloride, at the same concentration as in passage 22, was added to solution W plus vitamin B<sub>1</sub>. Growth, not quite so profuse as that obtained in solution W plus vitamin B<sub>1</sub> alone, took place. Evidently a failure of roots to grow in a solution with NH<sub>4</sub>Cl as the only source of nitrogen was not related to the toxicity of the ammonium salt.

It is concluded that excised tomato roots required for growth no other source of nitrogen than nitrates, and that nitrogen in the form of ammonia was not available under the conditions of our experiments. It appears probable that unlimited growth of tomato roots would occur with no other source of nitrogen than nitrates.

Was the amount of nitrogen in WHITE's solution or in solution W

a limiting factor for the growth of tomato roots in our experiments? We believe it was not. The increased growth obtained on the addition of boron and zinc to the minerals of WHITE's solution would suggest that nitrogen was not a limiting factor in that solution. The increased growth obtained through the addition of filter paper ash to solution W containing vitamin B<sub>1</sub> would suggest the same conclusion. If we assume 2.5% N in the dry matter of these roots (9), then the N of the nitrates in 50 ml. of WHITE's solution should permit the formation of about 60 mg. of dry matter. The maximum dry weight secured in 50 ml. of WHITE's solution was usually between 20 and 30 mg. (in one instance 43 mg.). We are inclined to believe that growth was limited by accessory mineral elements present in inadequate amounts as contaminants in WHITE's solution. The limiting action of supplementary mineral elements was more marked when the yeast was replaced by vitamin B<sub>1</sub> because of the elimination of the supplementary elements in the yeast ash.

LIGHT AND DARKNESS.—Limited experiments with roots grown in the dark have confirmed WHITE's report (31) that light is unnecessary for continued growth of excised tomato roots. Roots grew in successive passages in the dark in WHITE's solution.

VARIABILITY IN GROWTH.—Throughout these experiments considerable variability was noted in the growth of excised tomato roots under similar conditions. One root might develop several times as much as another, even though both were grown in aliquots of the same solution for the same period of time and under the same conditions of light and temperature. The variability to which we refer is not that resulting from complete failure of a particular root fragment to grow, although we have included some such instances in the data. Even when both roots under consideration grew, one might develop much better than the other. Examples of this may be found by examining ranges in dry weights given in the tables.

ROBBINS and WHITE (16) were of the opinion that the somewhat similar results found with excised roots of corn might be because of its heterozygous condition. Such an explanation would not apply here. The excised tomato roots with which we have worked were from two clones, B and C, derived from two original roots. The variability referred to was noted in roots from each clone; further-

more no consistent differences were noted between the growth of roots from clone B or clone C under the same conditions.

We do not believe that the variability can be accounted for by differences in the solutions, contamination of glassware, or the environmental conditions under which the roots were grown. Aliquots of the same solution were used in any particular experiment. The glassware was carefully cleansed with chromic acid cleansing mixture and rinsed six times with tap water, three times with distilled water, twice with redistilled water, and dried by draining. A given series of flasks were placed together under the same light and temperature conditions.

We do not believe that injury in fragmenting or transferring can account for the variability. Injury might account for the failure of an occasional fragment to grow, although care was always used to cool the knives and needles employed in fragmenting and handling the roots. Injury would not seem responsible where one piece grew poorly and another well.

The character of the root fragment used as inoculum may be responsible, although this has not been demonstrated. Variability in development of pieces which appeared similar at the time of inoculation was noted. But even though two pieces appear much alike, they may have come from different parts of a root and may differ in relative proportions of various tissues and in chemical composition.

It is not probable that the number or proportion of growing points was significant. This conclusion followed from results of an experiment in which the growth of pieces without evident growing points was compared with that of those with one or more growing points. Both types grew and no difference in the extent of growth was noted.

Evidence accumulated during these experiments that the nature of the solution in which the root fragmented for inoculum grew may influence its later development. This effect, which we have called carry-over, is discussed in the next section. The examples of carry-over noted were not sufficiently great to account for the variability observed. Furthermore the inoculum for any particular set was, as a rule, taken from roots which had developed in solutions of uniform composition, and frequently from a single root.

**CARRY-OVER.**—When roots which were growing rapidly were used as sources of inoculum for an unfavorable medium, considerable

growth often occurred in the first passage; but subcultures in the unfavorable medium failed to grow. This carry-over effect was noted in a number of experiments. For example, some growth occurred when WHITE's solution without yeast was inoculated with rather large pieces of roots which had been growing in WHITE's solution. No growth could be detected when this same solution without yeast was inoculated with a root fragment from a root grown in a solution containing 0.001 gamma or less of vitamin B<sub>1</sub> per flask.

In passage 20, flasks of a solution composed of mineral solution and light brown sugar treated with charcoal were inoculated from an Eastman's maltose culture. The pieces of inoculum were rinsed in sterile mineral solution. The roots grew appreciably in this medium, which contained no yeast or substitute for yeast. The average dry weight for four roots was 1.0 mg., and the largest root weighed was 1.5 mg.

Results showed that pieces of excised roots, even when rinsed in sterile mineral solution, carried with them enough vitamin B<sub>1</sub> or substitute to produce appreciable growth, but not enough to maintain this growth through more than one passage.

VITAMIN B<sub>1</sub> AND THIAZOLE AS RELATED TO SULPHUR.—Vitamin B<sub>1</sub>, or its intermediate thiazole, was found to be essential for the growth of excised tomato roots. In addition to carbon, hydrogen, nitrogen, and oxygen, these compounds contain sulphur. Do they function as available sources of sulphur? To test this the roots were grown in WHITE's solution containing vitamin B<sub>1</sub> or thiazole but without inorganic sulphur. The MgSO<sub>4</sub> in WHITE's solution was replaced by MgCl<sub>2</sub>, and the Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> was replaced by FeCl<sub>3</sub>. The roots in the solutions containing vitamin B<sub>1</sub> or thiazole but lacking inorganic sulphur failed to grow. From these experiments it was concluded that the vitamin B<sub>1</sub> or thiazole used in the culture media did not replace the sulphur in the mineral solution.

### Discussion

Excised roots of a green plant, *Lycopersicon esculentum* Mill., were found to require for unlimited growth mineral salts, sugar, and thiazole (or vitamin B<sub>1</sub>) in addition to water and dissolved gases. These investigations have not entirely eliminated the possibility that other sources of carbon might be substituted for sugar and

other compounds might substitute for vitamin B<sub>1</sub> or thiazole. Furthermore it is conceivable that the requirements of excised tomato roots in liquid culture may not be the same as those of roots attached to the tomato plant and growing under natural conditions in an aerated soil. Nevertheless it is reasonable to assume from the results that the tomato root requires an external supply of both sugar and thiazole (or vitamin B<sub>1</sub>). If this assumption is correct, it would appear that the attached tomato root depends upon the top for both sugar and thiazole (or vitamin B<sub>1</sub>). Data on the synthesis of sugar by the tomato top, its translocation to the root, and its utilization are sufficient to form a clear concept of this relation. Similar information on vitamin B<sub>1</sub> or thiazole is lacking.

Why should the top of the tomato plant form thiazole (or vitamin B<sub>1</sub>) while the root does not? It would not appear to be a light relationship, since some fungi apparently form vitamin B<sub>1</sub> in the dark. It would not appear to be related necessarily to chlorophyll, since some fungi (which lack chlorophyll) form vitamin B<sub>1</sub> (5).

Where and how is thiazole translocated in the plant? It would be reasonable to assume that it is translocated through the phloem, although definitive evidence on this point is lacking. If it is translocated through the phloem, how significant in ringing or girdling experiments is interference with its movement? Experimenters in this field have hitherto confined themselves largely to a consideration of carbohydrates, nitrogen, and mineral salts.

Is thiazole, alone, necessary for the growth of the tomato root, or does the root actually require vitamin B<sub>1</sub>, synthesizing pyrimidine but little or no thiazole and forming the vitamin when supplied with thiazole? We are inclined to believe that the latter assumption is correct.

If the vitamin is required, how does it function? On this we have little information. In his work with *Phycomyces*, SCHOPFER has suggested that vitamin B<sub>1</sub> functions in nitrogen metabolism (24). His evidence is not entirely convincing, but his hypothesis offers one possibility. Suggestions have been made that vitamin B<sub>1</sub> is concerned with carbohydrate metabolism in animals, and some support has been accumulated for this hypothesis (27).

The extremely minute amounts which are effective, and the huge

ratio of dry matter formed to vitamin present, are intriguing facts which must be considered in any hypothesis dealing with the function of vitamin B<sub>1</sub>. These questions and many others are raised by our findings of the essential nature of thiazole (or vitamin B<sub>1</sub>) in relation to the growth of the tomato root.

In any event, our results and those of BONNER (1, 3), secured independently with excised pea roots, confirm the original hypothesis of ROBBINS, and of ROBBINS and MANEVAL. This hypothesis was that the failure of excised roots to make unlimited growth in solutions of mineral salts and sugar is because of the lack in the medium of some necessary material derived by the seedling root from the grain and exhausted during growth of the root. WHITE's conclusion (29) that our original experiments which indicated the existence of a "hormonal" factor must be explained in some other way is in error. He neglected to consider the materials included in the yeast extract which was added to the medium.

As already pointed out, the root (which contains no chlorophyll) depends upon the green top for the carbohydrates used in its metabolism. It appears that tomato roots are not highly specific as to sources of carbon which can be utilized. They grew well with dextrose, levulose, maltose, sucrose, and to some extent with cellobiose. Our experiments, however, did not include a sufficient variety of carbon sources to permit us to conclude much on the availability of different kinds of carbon compounds and the way in which they are assimilated. It appears clear from our results, especially with different samples of maltose, that in such work greater care than is usual should be given to the purity of the compounds used.

These experiments show that excised tomato roots are capable of using nitrates in the synthesis of their proteins. This is significant since it indicates that root cells in the green plant may not necessarily depend upon the top for organic nitrogen. While excised tomato roots appear capable of synthesizing protein from nitrates, this does not exclude the possibility that an attached root may derive organic nitrogen from the top and be benefited thereby. WHITE (33) and BONNER and coworkers (1, 2, 3) have reported a beneficial action on the growth of excised roots from the addition of amino acids to a solution of mineral salts, cane sugar, and vitamin B<sub>1</sub>. But we cannot



agree with BONNER and WHITE's statements that amino acids are essential or necessary for the growth of excised roots.

Since these cells are able to utilize nitrates, it might be anticipated that ammonia also would be available. Under the conditions of our experiments the excised roots were unable to grow with ammonium salts as the sole source of nitrogen. Could this failure be related to a deficiency in oxygen? It is possible that nitrates may be important in the respiration of tomato roots submerged in liquid cultures. No experiments which would clarify this question have been performed.

The synthetic media, composed of mineral solution, pure sucrose, and vitamin B<sub>1</sub> or thiazole, have proved sufficient for growth through a number of passages with no consistent lessening of growth. The growth of excised roots is not so rapid in these media, nor does it continue so long in a single passage as it does in certain other non-synthetic media, such as those containing light brown sugar or malt flour. The conclusion might be drawn that the synthetic media were not adequate, because better growth occurred in other solutions. Everything necessary for continued growth was supplied by these synthetic solutions, however, and there is little reason to doubt that growth comparable in amount to that obtained in the "better" media could be obtained if fresh supplies of the synthetic media were furnished at frequent intervals.

In spite of the high purity of the cane sugar used in our experiments, a purity indicated by an ash content of less than 0.0001%, it must be remembered that cane sugar is a natural product and may contain traces of organic impurities. Although we believe a solution of mineral salts, sugar, and vitamin B<sub>1</sub> or thiazole is adequate for unlimited growth of excised tomato roots, a complete demonstration must wait experiments in which some synthetic carbon compound is successfully substituted for those derived from natural sources.

More attention should be devoted to the rarer mineral elements as limiting factors in the growth of excised roots. It is likely that the superiority of Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> over FeCl<sub>3</sub> is not associated with the Fe, as believed by WHITE (28), but with the mineral contaminants present to greater degree in the Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>. Also, the factor limiting growth of excised tomato roots in WHITE's solution may be the amount of one or more of the rarer elements. It is unfortunate that supplies of high-

ly purified chemicals are not available commercially for physiological work. Until they are, and until there is a more general realization of the importance of traces, conclusions on the mineral nutrition of excised roots will be faulty.

### Summary

1. The possibility of unlimited growth of excised tomato roots in WHITE's solution composed of mineral salts, sugar, and yeast extract was confirmed.

2. Each of the three parts of this medium was essential.

3. The essential nature of the yeast extract was because of its vitamin B<sub>1</sub> or vitamin thiazole content.

4. Unlimited growth of excised tomato roots appeared possible in a solution of mineral salts, cane sugar, and vitamin B<sub>1</sub> or thiazole.

5. The following compounds in the amounts used did not substitute for vitamin B<sub>1</sub> or the vitamin thiazole in the growth of excised tomato roots: indole(3)acetic acid, yeast ash, vitamin C, pantothenic acid, vitamin G (B<sub>2</sub>), asparagine, cysteine hydrochloride, inositol, urea, pimelic acid, hydrolyzed nucleic acid.

6. Neo-peptone, malt flour, and certain samples of maltose at suitable concentrations substituted for yeast, probably because of their vitamin B<sub>1</sub> or thiazole content.

7. The growth of excised tomato roots was affected by extremely small amounts of vitamin B<sub>1</sub>. The effect of 0.00000001 mg. (1 part in 40,000,000,000,000 parts of culture solution) was observed. It is believed, however, that the presence of 0.1 gamma or more is necessary for unlimited growth.

8. Tomato roots grown in a solution supplemented with thiazole alone synthesized the pyrimidine intermediate of vitamin B<sub>1</sub>.

9. Vitamin B<sub>1</sub> did not replace inorganic nitrogen nor inorganic sulphur for tomato roots.

10. Excised tomato roots apparently required no other source of nitrogen than nitrates for unlimited growth.

11. Cane sugar, glucose, levulose, maltose, or cellobiose were available carbon sources for excised tomato roots.

12. The contaminants in "pure" sugars markedly affected the growth of excised tomato roots.

13. WHITE's mineral solution was not adequate for best growth of tomato roots.

14. The addition of boron and zinc improved WHITE's mineral solution.

15. Certain samples of filter paper and of filter paper ash improved WHITE's solution.

16. A modified Pfeffer's solution with 2.5 p.p.m. of  $\text{Fe}_2(\text{SO}_4)_3$  instead of 0.8 p.p.m.  $\text{FeCl}_3$  was superior to WHITE's mineral solution when yeast extract was replaced by vitamin  $\text{B}_1$ .

17. Excised tomato roots did not require light for unlimited growth.

18. Variability in the growth of individual roots under the same conditions may have been associated with differences in the composition of the fragments used as inoculum.

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# CROSSING-OVER, FRAGMENTATION, AND FORMATION OF NEW CHROMOSOMES IN AN ALLIUM SPECIES HYBRID<sup>1</sup>

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(WITH FOURTEEN FIGURES)

## Introduction

The chromosomes of *Allium* species, because of their large size and conspicuous constrictions, are exceedingly favorable for cytological studies. As pointed out by LEVAN (18), they may be grouped under three general types: those with median insertions which divide them into arms less asymmetrical than 2:3; those with arms showing greater asymmetry than 2:3; and the subterminally constricted ones in which the shorter arm is less than one-fourth the length of the entire chromosome.

In an earlier paper (9) the writers discussed some cytological phases of a hybrid between *Allium cepa* L. and *A. fistulosum* L. The *A. cepa* parent was a strain of Yellow Globe Danvers (15-108-1), inbred two generations, and the *A. fistulosum*, a strain designated as 37-1. This paper is concerned almost entirely with the irregularities that occur in the same hybrid. Since *A. fistulosum* has been shown to be highly resistant to onion smut (*Urocystis cepulae* Frost), pink-root (*Phoma terrestris* Hansen), and thrips (*Thrips tabaci* Lind.), it was felt that a cytological study of the *cepa*×*fistulosum* hybrid should throw some light on the possibility of securing recombinations of the genes of the two species.

Recently LEVAN (19) has also published on the cytological behavior of a *cepa*×*fistulosum* hybrid, in which the commercial variety Braunschweiger was crossed with *A. fistulosum*. In some particulars the cytology of our hybrid closely parallels that of LEVAN's, while in others there are considerable dissimilarities. Among these, the

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most noteworthy are: the considerably higher frequency of first metaphase bivalents; almost complete absence of first metaphase polyvalents; high frequency of fragments and chromatids with two insertion regions, and greater fertility when used in backcrosses. These differences were noted by LEVAN and attributed by him to a closer relationship between our parental forms. That this is probably true is indicated by the even greater fertility of more recent hybrids made by us between other varieties of *cepa* and the same form of *fistulosum*. Likewise, additional hybrids of *fistulosum* with still other varieties of *cepa* have proved more sterile. Cytological observations of these have not yet been made, with the exception of a few slides from one between the *cepa* variety, Australian Brown, and *fistulosum*. MAEDA (24) has also recently published on the chiasmata of *cepa*, *fistulosum*, the  $F_1$ ,  $F_2$ , and first backcrosses. He also reports the occurrence of fragments but makes no mention of chromatid bridges.

### Methods

All the photomicrographs are from aceto-carminic smears made as described in an earlier paper (9). The post-meiotic chromosomes are from gentian violet smears prepared according to the method of LEVAN (20). The photomicrographs were taken with a Zeiss photomicrographic camera, on a Zeiss microscope with 15 $\times$  ocular and 40 $\times$  dry objective. The magnifications are *ca.* 600 and there has been no reduction in reproduction. The camera lucida drawings were made with a Zeiss microscope with 15 $\times$  ocular and 90 $\times$  1.4 apochromatic oil immersion objective, giving a magnification of 2100 at table height. Reductions are given with each figure.

### Investigation

#### MEIOSIS IN THE HYBRID

The somatic chromosome number in both *Allium cepa* and *A. fistulosum* is sixteen. The frequency of metaphase pairing in the hybrid was variable, as shown in table 3 of an earlier paper (9). There was a preponderance of complete pairing, however, the pollen mother cells with eight bivalents comprising slightly over 72 per cent of the 130 examined. In only one instance was a trivalent observed at first metaphase (fig. 3A). This situation differs strikingly

from LEVAN's hybrid, in which only 8 per cent of 100 cells studied contained eight bivalents. He also found in these cells three trivalents, two tetravalents, and one hexavalent.

The occurrence in our hybrid of chromatids with two insertion regions is fairly common in first anaphase and subsequent phases of meiosis. There is also a high frequency of fragments. The subsequent description of meiosis in the *cepa* × *fistulosum* hybrid is concerned mostly with the appearance and behavior of these irregularities and their bearing on formation of morphologically new types of chromosomes.

LEPTOTENE.—At leptotene it was impossible to observe any differences between the hybrid and parental forms. As reported in an earlier paper, the leptotene chromosomes appeared to be unsplit, but in the light of recent investigations by KOSHY (16), RUTTLE and NEBEL (33), and DERMEN (8), it may be that a different technique might have revealed a more complex structure.

ZYGOTENE.—At this phase of meiosis in the hybrid (fig. 1A) the first indications of irregularities were observed. It is very difficult to analyze them because of chromosome clumping. There are, however, many multivalent associations which give rise to considerable entanglement among the chromosomes. Frequently two chromosomes paired for a short distance separate, each then pairing with another. As a result unpaired areas such as shown at *a*, figure 1A, are rather common at this stage. This is in strong contrast to both *fistulosum* and *cepa*, where similarly unpaired chromosome sectors were not observed. LEVAN (19) reports the same situation in his hybrid.

PACHYTENE.—At pachytene, multivalent associations were also common. Occasionally a cell was observed that closely resembled the condition in "sticky chromosome" plants of *Zea mays* as described by BEADLE (2). A later stage (fig. 2E) closely resembles his anaphase group of plate 1, figure 30. It is possible that some of the apparent multivalent associations at the pachytene and earlier stages are not actual pairings but chromosome overlaps. This condition is undoubtedly accentuated by the flattening of some of the pollen mother cells; but even accepting this possibility, there are many unquestionable multivalent associations. The situation is



very similar to pachytene pairing in a diploid *Allium schoenoprasum*, following exposure of umbels to x-ray treatment (22). The *schoenoprasum* flowers were of various sizes, so that all phases of meiosis were subjected to treatment. Pollen mother cells were fixed daily for 4 days after irradiation. One plant which was exposed to Roentgen rays for only 15 minutes showed the following on a slide made on the third day following treatment: "Very clear pachytene stages of this specimen frequently showed examples of abnormal pairing of the threads. Trivalent and quadrivalent configurations occurred, as well as bivalents with inversions." These irregularities in *schoenoprasum* strongly suggest translocations which are known to occur rather frequently as a result of irradiation.

In the *cepa* × *fistulosum* hybrid described in this paper a few fragments were found at pachytene. They were completely separated from the remainder of the chromosomes, and because of their small size could not have been univalents or foldbacks. While univalents were also common, they could usually be identified because of their larger size. Classification as a fragment is necessarily very broad, since any piece of a chromosome regardless of size may be considered one. In this paper only those clearly smaller than any of the parental chromosomes are classed as fragments. They may or may not have an insertion region. Undoubtedly the actual number, when interpreted in the broad sense, was much higher.

**DIPLOTENE.**—At diplotene multivalents are found less frequently, indicating that dissociation of earlier pairing has taken place. In many instances the chromosomes form a tangled mass (fig. 1B–F). Unfortunately the insertion regions of *Allium* chromosomes are not recognizable in early meiotic stages. Because of this it cannot be determined whether they are always exactly opposite each other in paired chromosomes. The size and morphology of the chromosomes of the two species are so similar, however, that such conditions as depicted at *a*, figure 1D, where in two bivalents the end of one chromosome extends beyond the other, indicate that insertion regions of paired chromosomes may sometimes not be opposite each other. Instances of this sort are common in this hybrid. In some cases a chromosome extends beyond the one with which it is synapsed and pairs with a third. This is true of the lower of the two in *a*, figure



FIG. 1.—Multivalent associations at zygotene and diplotene: *A*, unpaired threads at *a* in zygotene; *B*, *C*, *D*, *E*, diplotene showing entanglement of chromosomes forming multivalent associations.

1*D*. Another configuration seen occasionally is that shown at *b*, figure 1*D*. The loop may be the result of an inversion. At *a*, figure 1*F*, the loop suggests a duplication. In figure 2*B*, *C*, and *D*, multivalent associations are very clear. None of these irregularities were observed in either *cepa* or *fistulosum*.

The occurrence of fragments (*f*) at diplotene is shown in figure 2*A–D*. They are undoubtedly fragments, size alone precluding any possibility of their being univalents. On the same basis, the possibility of their being foldbacks can also be eliminated. Lest they may have been caused by mechanical injury as a result of the method of making slides, a great number of observations were made on similarly prepared material of the parent forms of *Allium cepa* and *A. fistulosum*. No fragments were found in *A. fistulosum* and but one in *A. cepa*. In the latter instance it occurred at late first anaphase and was associated with a chromosome bridge. These observations indicate that fragments are typical for this hybrid.

DIAKINESIS.—By the time diakinesis is reached, the chromosomes are well spaced and can readily be determined as univalents or bivalents. The time required for this phase of meiosis is probably comparatively short, since the number of diakinesis figures observed was considerably smaller than any other stage. Polyvalent associations are rare, only one being observed in seventy-five cells. It is shown in *a*, figure 2*F*. Here each end of a chromosome is paired with two others that lie some distance from each other. As a result the connecting chromosome is pulled out across the cell. The blurred spot at the right was caused by extraneous material on the under side of the cover slip. The loop at *b* is a type of configuration that occurs rather frequently. It is essentially the same configuration as shown at *b*, figure 1*D*.

FIRST METAPHASE.—At metaphase the chromosomes in 232 cells, with the exception of the one trivalent shown at *a*, figure 3*A*, were either univalents or bivalents. The bivalents form one of two general types of configurations, either rods or rings (fig. 3*B*). All the chiasmata become terminal as in *A. cepa*, and the hybrid metaphase plates, in which irregularities do not occur, are almost identical with those of *A. cepa*.

The bivalents of the hybrid and *A. cepa* differ markedly from

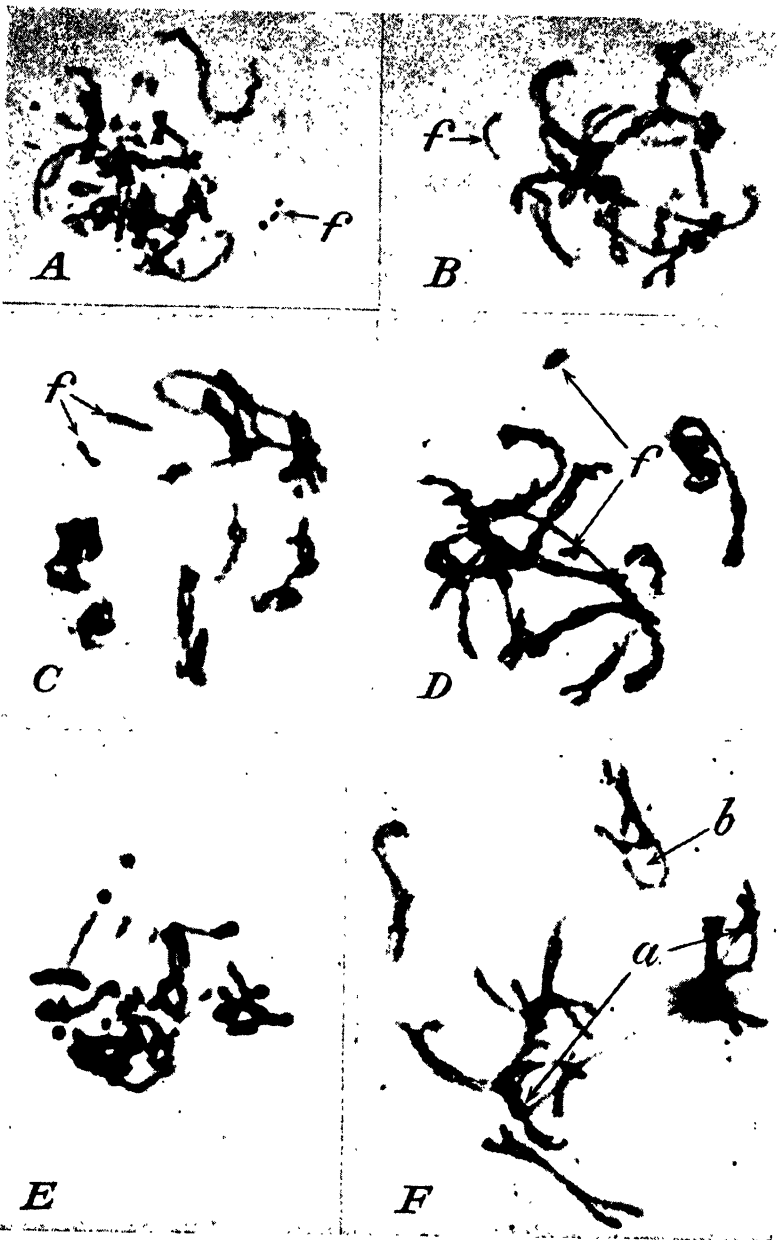


FIG. 2.—A, B, C, D, showing fragments (*f*); E, entanglement very similar to “sticky gene” situation in *Zea mays*; F, multivalent association at diakinesis.

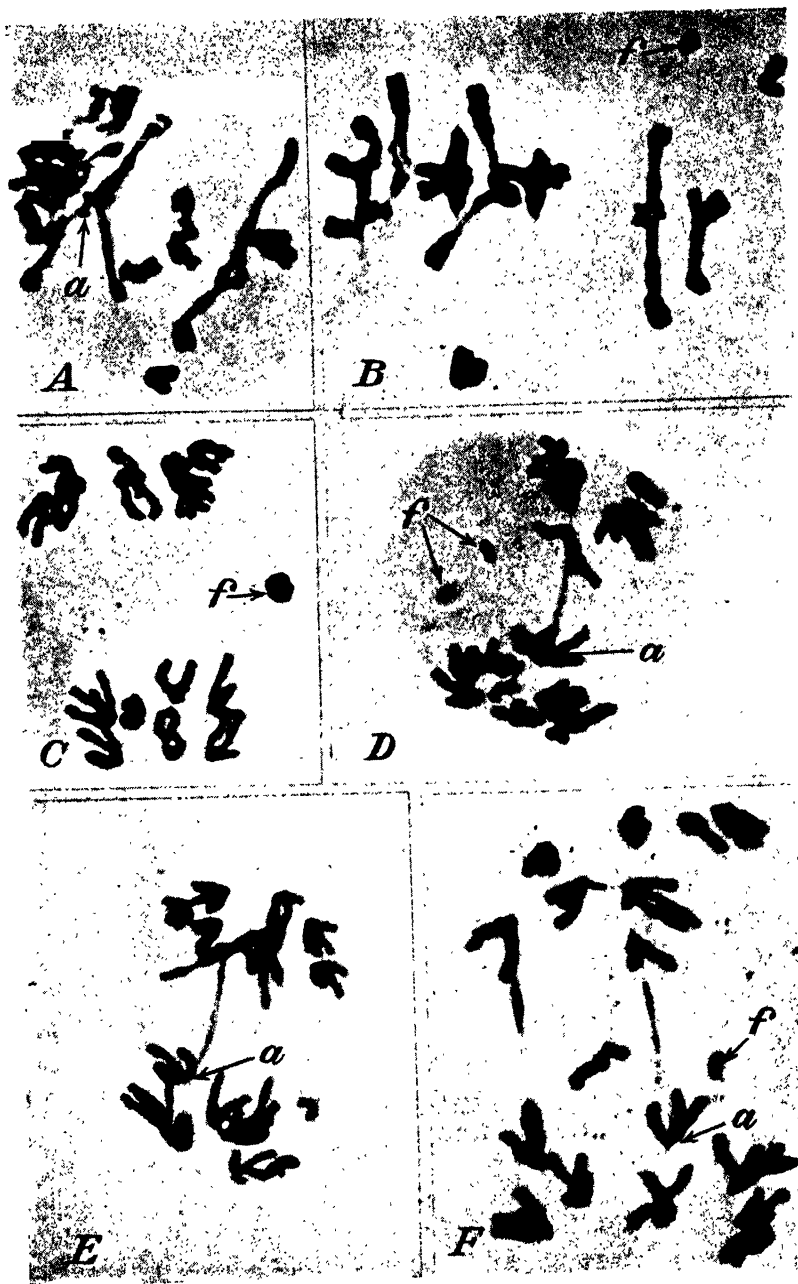


FIG. 3.—*A*, trivalent at first metaphase; *B*, fragment and univalents at first metaphase; *C*, fragment at first anaphase; *D*, *E*, *F*, bridges at first anaphase. Note three free arms at each pole.

those of *A. fistulosum*. As shown by ISHIKAWA (14), LEVAN (21), and EMSWELLER and JONES (9), this variation between the species is caused by differences in location of chiasmata at first metaphase. In *A. cepa* at early metaphase they are randomized, while in *A. fistulosum* they are localized at the insertion region. In *cepa* they later become terminal, in *fistulosum* they do not. EMSWELLER and JONES (10) recently presented data which indicated that localization in *A. fistulosum* was probably controlled by a recessive gene. LEVAN (18) has found a somewhat similar situation in *A. nutans*, there having appeared a plant with localized chiasmata in an inbred line of this species which normally shows only a random arrangement of the chiasmata at first metaphase.

At first metaphase there are many more cells containing fragments than at diplotene and diakinesis. A metaphase group with a fragment (*f*) is shown in figure 3*B*. There are seven bivalents, two univalents, and one fragment. Fragments of this type are relatively easy to determine. It is only when they are almost as large as a univalent that difficulties arise.

FIRST ANAPHASE.---The first anaphase figures may be grouped into two classes, those with chromosome bridges and those without. In figure 3*C* there are eight chromosomes at each pole and a fragment lying near the equatorial plate. This type of first anaphase was not uncommon; that is, there were many cells without bridges but containing one or more fragments.

First anaphase groups with one bridge are shown in figure 3*D* and *E*. Two bridges occur in *F*. In *D* and *F* there are unmistakable fragments, and in *E* to the right of the lower group of chromosomes there is a small mass of chromatin, which appears to be a univalent but may be a large fragment. At *a*, in each of these three figures, it can be seen that the bridge is simply a chromatid with two insertions, which are moving to opposite poles. At each end of the bridge a chromatid with one attachment is clearly seen held fast to the doubly constricted one at the insertion region. Close examination of these figures shows three free chromosome arms at each pole with the fourth stretched out to form a so-called bridge. Such a bridge then consists of three chromatids, two having one insertion each, while the third has two.

FIRST TELOPHASE.—A “normal” first telophase group is shown in figure 4*A*. There are no visible fragments in this cell, but in many similar cells they do occur, sometimes masked at one or both poles by the darkly staining group of chromosomes. In figure 4*B* two small fragments (*f*) can be seen at the left, and either a larger one or a univalent at the right (*a*). It is clear that separation of the latter is being completed.

The first telophase groups of figure 4*C* and *D* contain a chromosome bridge connecting the chromatin masses at each pole. In *E* there are two bridges and in *F* there are three. In *C* no fragments are found. It is possible, however, that one might be included in the deeply stained chromatin at either pole, or have disintegrated before this stage of division was reached. In *D* a small fragment occurs near the bridge. The two bridges in *E* are accompanied by at least one fragment, and there is another small one and possibly a dividing univalent in *F*.

Occasionally there occurs a cell containing a great number of fragments. One is shown in figure 5*A*. There are seven unquestionable pieces of chromosomes, and two others may be univalents but are probably large fragments. What appears to be the remnant of one bridge is seen at *a*.

FIRST INTERPHASE.—At first interphase, when fragments occur, the cell may or may not contain one or more bridges. In figure 5*B* there are five visible fragments and what may be the remnant of a bridge (*a*) jutting out from the lower nucleus. In *C* there is one fragment and probably a univalent. The bridge in *D* was not accompanied by a fragment, whereas in *E* a small one is present.

The behavior of chromosome bridges during the first interkinesis was given considerable study. In figure 5*D* and *E* the outlines of intact ones can readily be seen. The chromatid with two insertion regions has persisted as a connection between the daughter interkinetic nuclei. Within each nucleus the identity of the chromosomes is almost obliterated. In slightly earlier stages (fig. 4*C–F*) the bridges are intact and stain as deeply as the telophase chromosomes at each pole. In other first telophase cells (figs. 5*F*, 6*A*, *B*, *C*), however, the bridges do not stain so deeply and do not extend completely across the cell as a connection between the newly formed daughter

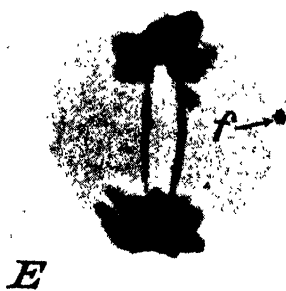
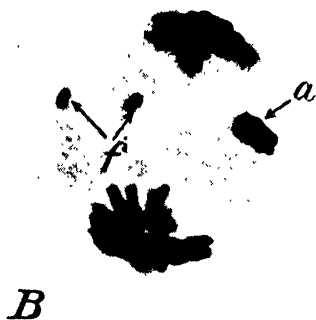


FIG. 4.—First telophase: *A*, normal figure without bridges or fragments; *B*, two fragments and univalent; *C*, chromatid bridge but no visible fragment; *D*, chromatid bridge and fragment; *E*, two chromatid bridges and one visible fragment; *F*, three chromatid bridges and one visible fragment.



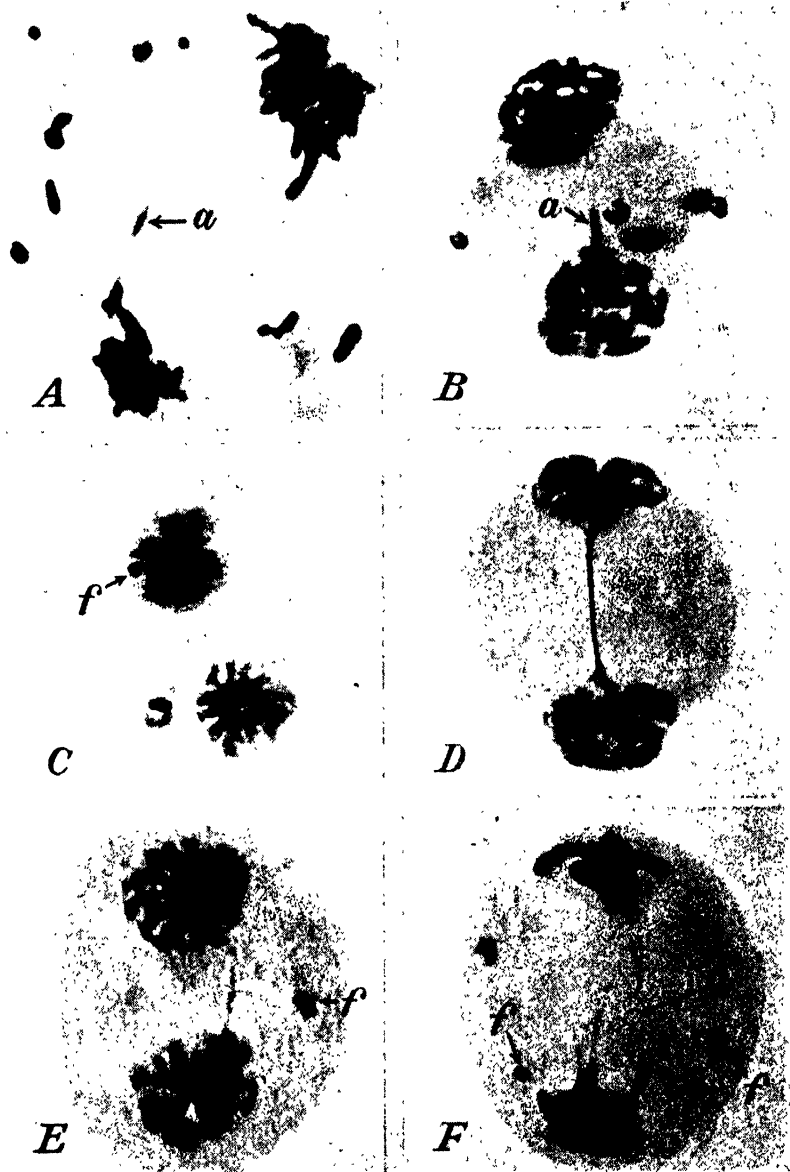


FIG. 5.—A, late first telophase showing numerous fragments and remnant of chromatid bridge; B, first interphase showing five visible fragments and remnant of bridge; C, fragment and univalent but no bridge; D, chromatid bridge but no visible fragment; E, disintegrating bridge and fragment; F, remnants of three bridges showing disintegration of central portions.

nuclei. In 6*B* and *D* a piece of a chromatid can be seen midway between the new daughter nuclei. The remnant of a bridge projects from each nucleus in *D*, and undoubtedly the piece of chromatid (*a*) was originally a part of this bridge. The cytological evidence indi-

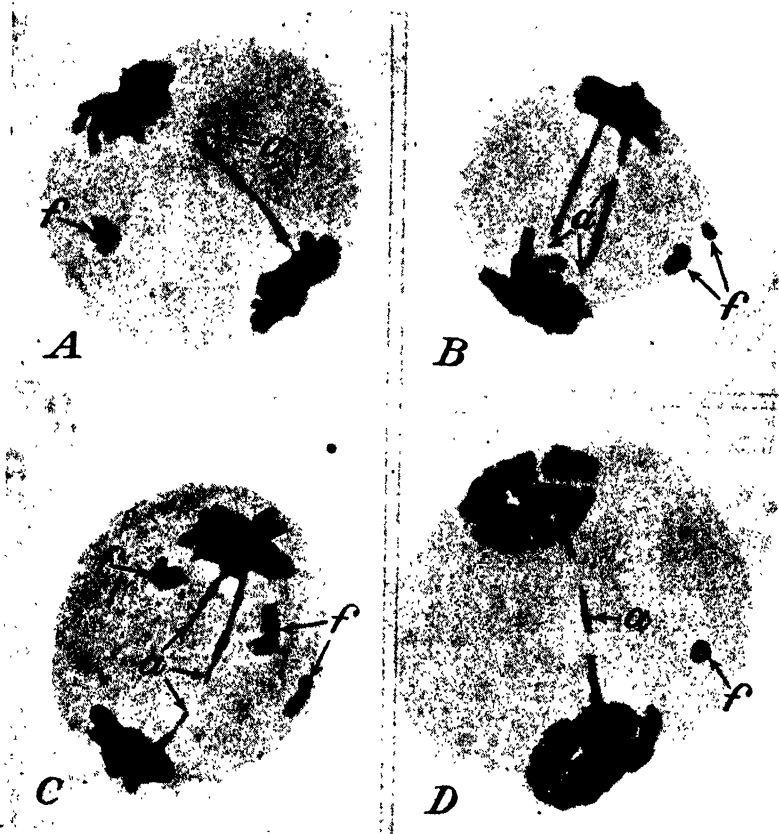


FIG. 6.—*A*, chromatid bridge disintegrating near upper nucleus; *B*, two bridges, one freed at one end, the other at both ends; *C*, two bridges, one almost completely lost, second with small portion lost near lower nucleus; *D*, first interphase with bridge "broken" at two points, leaving piece out in center.

cates that a bridge may become disconnected at one or more points between the daughter nuclei. There is no indication that cytokinesis has been initiated as yet, therefore it cannot be responsible for the breaks in the bridges.

**SECOND METAPHASE.**—A second metaphase is shown in figure 7*B*. The pollen mother cell wall has not been ruptured and the daughter cells are not flattened out. Chromosomes with two insertion regions were not observed with certainty in second metaphase groups, but wider search should have discovered some, since the occurrence of bridges at second anaphase indicates that they sometimes reach one or the other pole.

**SECOND ANAPHASE.**—At second anaphase the bridge configurations are distinctly different from those of the first division. They are formed by one chromatid which has two insertion regions, and the two sister chromatids with but one are no longer attached to it, having been released by separation of the insertion regions, thus completing the mitotic split. This condition is shown at *a* in figure 7*C*. The sister anaphase cell did not have a bridge, so the one shown in the photomicrograph was freed from it in order to be more easily flattened for photographing. In the cell shown there are seven chromosomes at each pole. The bridge is composed of a chromosome with two insertion regions, which were so oriented at this division that they went to opposite poles. A comparison of this figure with figure 3*D*, *E*, and *F* will demonstrate the differences described.

In figure 7*A*, cytokinesis has occurred and the second anaphases are actually in separate cells, although they were both held within the pollen mother cell wall, which was ruptured, allowing the chromosomes to be flattened out. Two fragments are included within one of the new cells and three within the second. Some of the configurations at the left are certainly suggestive of chromatid bridges.

A very interesting second anaphase group is shown in figure 7*D*. There are seven fragments and seven bridges, three of which are interlocked. Cytokinesis has not occurred and the interlocked chromatids with two insertion regions have persisted into the second division. The probability of this anomaly is certainly very low, since it represents the formation of seven chromatids with two insertion regions out of a total of eight possibilities. There must also have occurred an interlock of two first anaphase chromatids, each of which had acquired two insertion regions. The situation is shown more clearly in the diagrammatic drawing of figure 8. There must

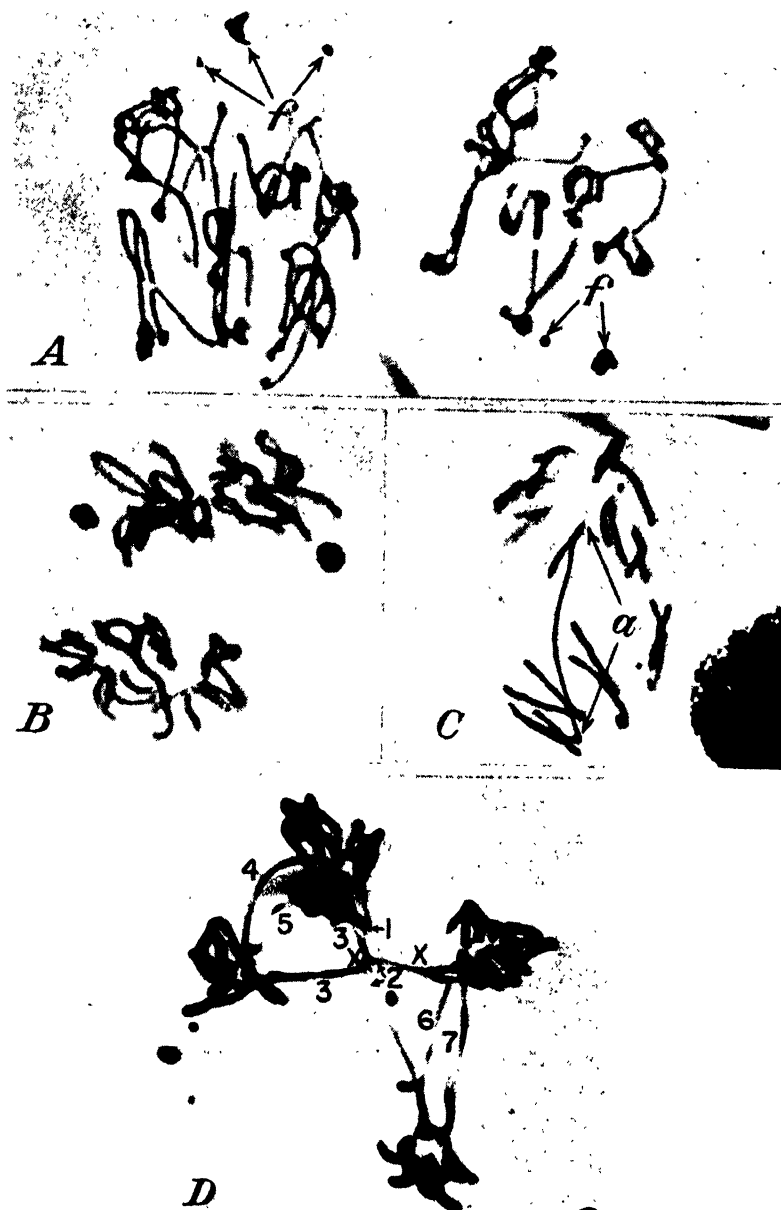


FIG. 7.—*A*, second metaphase showing fragments (squeezed out of p. m. c. wall); *B*, same stage in p. m. c. wall; *C*, second anaphase showing structure of chromatid bridge at this stage of meiosis (note that it is simply a single chromatid); *D*, complex second anaphase group showing interlocking of chromatid bridges (see fig. 8).

have been at least seven chromatids with two insertions at first metaphase, and there would have been but one bridge at first anaphase had interlocking not occurred. In the diagram of first telophase (fig. 8) the only true bridge is numbered 1. It is interlocked with chromatid 2 at  $X$ , and chromatid 2 is also interlocked with 3 at  $X'$ . The two insertions of 2 were oriented to one pole and those of 3 to the other at first anaphase. These chromatids would not have appeared at this division if the interlock at  $X'$  had not occurred. There might have been some difficulty at  $X$ , but it is likely that chromatid 2 would have slipped along 1 and been included in the

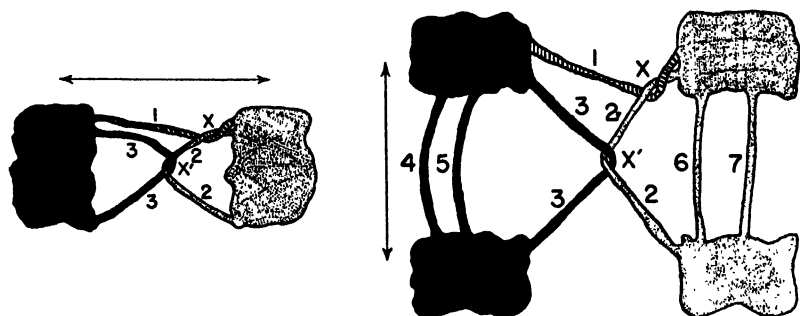


FIG. 8.—Diagrammatic sketch of *D* in fig. 7: first anaphase at left and second at right. Arrows denote planes of divisions.

new daughter nucleus. The other four bridges, 4, 5, 6, and 7, did not appear until the second anaphase, which was oriented as shown by the arrow. Cytokinesis did not occur following the first division, otherwise the interlocked chromatids would not have persisted. Possibly such an unusual condition prevented a normal first interkinesis and hastened the second division.

**SECOND TELOPHASE.**—A second telophase without bridges is shown in figure 9*A*. There is at least one and perhaps two fragments. The same stage with a bridge in each anaphase group is shown in 9*B*. Fragments were not visible in this figure, but might have been included in any of the four masses of chromatin or have disintegrated by this time. Two fragments in *C* are excluded from the telophase groups, and two bridges are visible at the right.

The behavior of bridges at the second telophase is essentially the same as at the first division. Some persist for a long time and show all the conditions as described earlier for the first division.

SECOND INTERPHASE.—A second interphase is shown in figure 9*D*. At least three fragments are visible. In this instance all three are outside the nuclei and may form micro-nuclei. At this stage also the remnants of bridges may sometimes be seen jutting out from

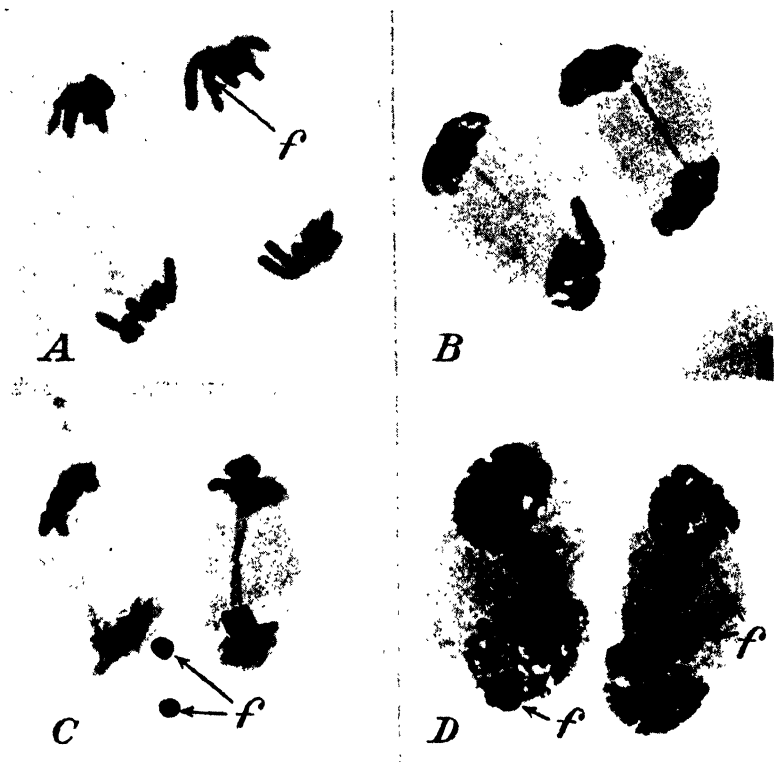


FIG. 9.—*A*, second anaphase showing one visible fragment and no bridges; *B*, bridge in each division but no visible fragments; *C*, one bridge at left and two at right, also two fragments; *D*, second interphase with three visible fragments and no bridges.

daughter nuclei. Intact bridges were not observed at second interphase. In many instances fragments become included in the second interphase nuclei and persist into the microspores.

#### POST-MEIOTIC CHROMOSOMES

The first post-meiotic mitosis in *Allium cepa* and *A. fistulosum* can be studied with comparative ease. The division takes place soon

after the four microspores are released from the microsporocyte. When the material is favorable it is possible to find many excellent metaphases on one slide. It was more difficult, however, to find good figures in the hybrid.

A count of the chromosomes in eighty-seven dividing microspores is summarized in table 1. There were seven different classes, the lowest number of chromosomes being six and the highest sixteen. Those with eight comprised the largest class, while those with nine

TABLE 1  
CHROMOSOME NUMBER OF MICROSPORES, AND BIVALENT  
FORMATION IN THE HYBRID

NO. OF CHROMO- SOMES IN MICROSPORE	FRE- QUENCY	PER- CENT- AGE	MICRO- SPORES WITH FRAG- MENTS	PER- CENT- AGE	TYPE OF PAIRING	FRE- QUENCY	PER- CENT- AGE
6.....	2	2.28	0	.....	.....	.....	.....
7.....	1	1.14	1	.....	.....	.....	.....
8.....	59	67.81	10	16.94	8 <sub>II-0I</sub>	94	72.30
9.....	15	17.24	4	26.66	7 <sub>II-2I</sub>	24	18.46
10.....	6	6.89	2	33.33	6 <sub>II-4I</sub>	9	6.15
11.....	1	1.14	1	.....	5 <sub>II-0I</sub>	1	0.76
.....	.....	.....	.....	.....	4 <sub>II-8I</sub>	2	1.53
16.....	3	3.42	0	.....	.....	.....	.....
Totals.....	87	.....	18	.....	.....	130	.....

were next numerous. There were three giant pollen grains with sixteen chromosomes which were spherical in shape, whereas the haploid and aneuploid types had the appearance of a slightly flattened ellipse. No observations were made on the mode of origin of these large pollen grains. It is interesting to note that microspores with less than the haploid number are able to survive thus far and to appear normal in every respect except chromosome number. It is not known whether such microspores can function, but the evidence on this point is meager. As noted earlier (9), the hybrid is almost completely egg sterile, but slightly pollen fertile. The backcrosses to *A. fistulosum* and *A. cepa* so far studied have all contained sixteen chromosomes. The number of backcross plants examined has not been large, however, and the data, although highly suggestive,

cannot be accepted as conclusive evidence that heteroploid microspores are not viable.

Included in table 1 are data on frequency of bivalent formation in the hybrid. The three most frequently occurring numbers are: eight bivalents, seven bivalents plus two univalents, and six bivalents plus four univalents. This is interesting when compared with the situation in the microspores. Here the three largest classes are eight, nine, and ten chromosomes. The three largest groups, both as to bivalent formation and chromosome number, are also expressed in percentage and there is close agreement between these figures. The microspores with eight chromosomes may arise following any type of pairing, and a higher percentage than actually found would not be unusual. There should also be approximately as many microspores with six and seven chromosomes as with ten and nine. Their absence is to be expected, however, since in general, reduction of chromatin in gametes and zygotes has greater lethality than addition.

In comparing the morphology of the chromosomes of *Allium cepa* with those of *A. fistulosum*, EMSWELLER and JONES (9) used the ratio of the length of the short to the long arm to secure an index number for each chromosome.

Twelve dividing microspores in the hybrid were selected for a critical study of the chromosomes they contained. They were chosen primarily because nearly all the chromosomes were flattened out, which is absolutely essential if arm measurements are to have much value. Since the frequency of bivalent formation in the hybrid was so high, it is reasonable to suspect that considerable crossing-over has taken place. As a result there have been formed several new chromosome types, which are shown in figures 10 and 11. In every instance the length of each arm was determined by measuring metaphase chromosomes of the first microspore division projected upon a sheet of paper with a Zeiss microprojector. The measurements of the chromosomes of *A. cepa* and *A. fistulosum* represent the average from eleven and nine cells respectively in which all the chromosomes were well flattened. The measurements from the hybrid represent single microspores, of course, and in some instances all the chromosomes of a cell were not ideally spaced or flattened. Each chromo-



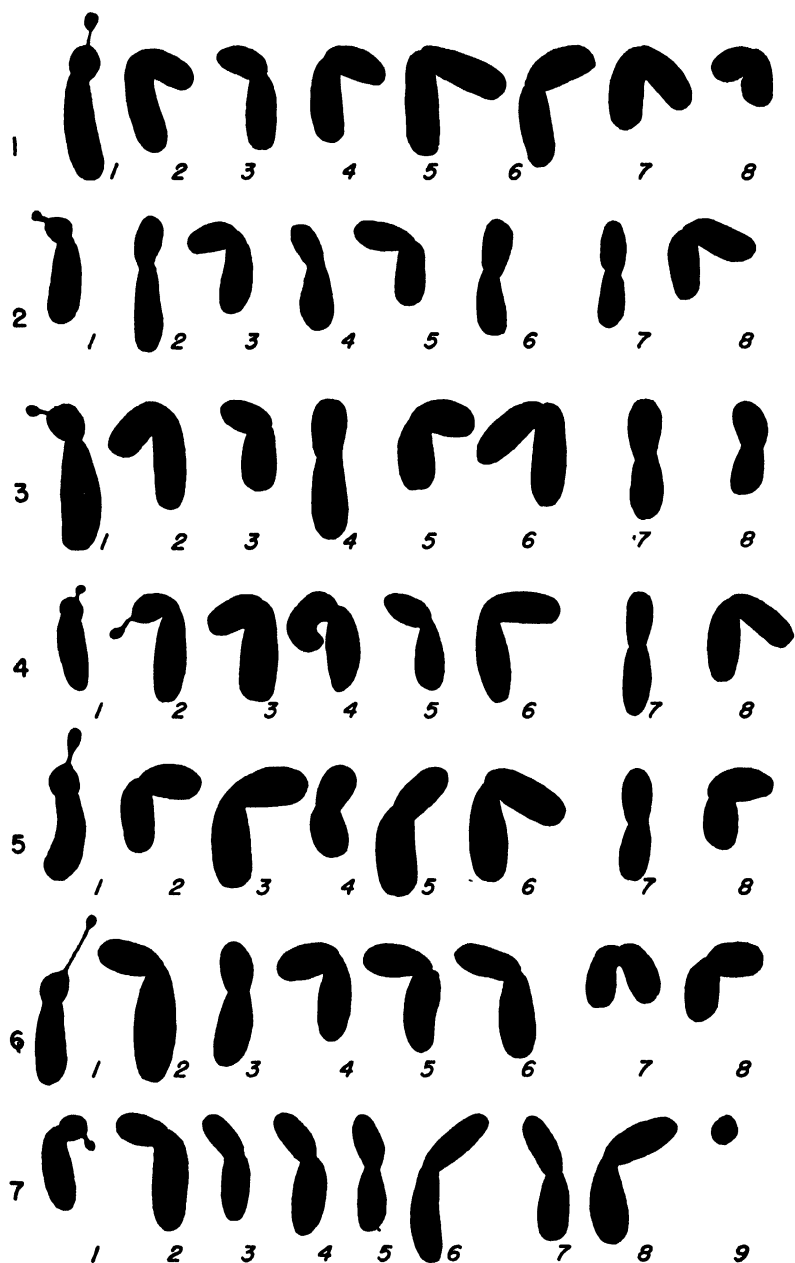


FIG. 10

FIGS. 10, 11.—Post-meiotic chromosomes in *Allium cepa*, *A. fistulosum*, and their hybrid.



FIG. 11

some, however, was measured at least fifteen times and the mean computed. The ratio of the short to the long arm was calculated and the data are recorded in table 2. In figures 10 and 11 the chromosomes are arranged and numbered in the order in which they appear in table 2. The one with the most nearly terminal constriction is at the left and the most nearly median at the right. The arrangement is not intended to signify homologies.

The morphology of the chromosomes in the microspores of the hybrid indicates that crossing-over was rather frequent. Unless both are changed proportionately, any modification in length of chromosome arms of course will result in a change in the ratio of the short to the long. Table 2 in conjunction with figures 10 and 11 indicates that such modifications have occurred. As shown in an earlier paper (9), the chromosomes of *A. cepa* and *A. fistulosum* may be placed in three groups, according to the location of the insertion region. There are in each species four median chromosomes, three submedian, and one subterminal, which has a satellite on the proximal arm.

In the description of each hybrid microspore, only the more easily distinguished new chromosomes are discussed. It is very probable that chromosomes other than those noted here were also modified in shape, but the changes are so slight that they could not easily be identified.

Microspores 1 and 2 are *cepa* and *fistulosum* respectively. In microspore 3, which is from the hybrid, there are five median, two submedian, and a satellited subterminal chromosome. Included are three large chromosomes, 1, 4, and 6, which are probably new types. The satellited chromosome resembles that of *cepa*, but the length of the distal in proportion to the proximal arm is greater and the index number accordingly reduced. Chromosome 4 is about the same length as *cepa* 5 and *fistulosum* 2, but its index number places it between them as to relative length of arms. Number 6 has its insertion more median than *cepa* 5 or 6, and is probably too long for *cepa* 7 or 8.

In microspore 4 there are two satellited chromosomes. It is the only instance in which two such chromosomes occurred in the same genom. Very probably this microspore arose from a pollen mother

cell in which not more than seven bivalents were formed, with the satellited chromosomes remaining unpaired and migrating to the same pole. It appears from table 2 that the satellited chromosomes in the hybrid microspore are shorter than in the parent species. Since even the same chromosome is known to vary in length in various cells of the same plant, these differences are probably of no significance. The index numbers indicate that chromosome 1 of the hybrid microspore is the *fistulosum* satellited chromosome and that number 2 is the unmodified *cepa* one. The deviation of number 1 from *fistulosum* (0.252–0.248) is within the range secured when nine *fistulosum* satellited chromosomes in as many microspores were measured. The actual figure for chromosome 1 of *cepa* was 0.3661, and for the second satellited chromosome of microspore 4 it was 0.3665. This is also well within the range secured from index numbers of eleven *cepa* satellited chromosomes. In addition to the evidence of index numbers, a difference in diameter of the two chromosomes also exists comparable with that between the parental chromosomes. This condition was noted by LEVAN (19). In his hybrid, the *fistulosum* chromosomes had a smaller diameter than *cepa*. Chromosome 1 is not so broad as 2, which fact further identifies it as the *fistulosum* satellited chromosome.

In genom 5 there are several deviations from parental chromosome types. The satellite is elongated and larger than that of either parent, and all the other chromosomes have median or nearly median insertions. The index numbers of 2, 3, 4, 5, and 6 are very similar and it would be difficult to distinguish between 2 and 4 and between 3, 5, and 6. Both 7 and 8 have fully median insertions, and certainly one is a new type since there was only one perfectly median chromosome in the hybrid. This was number 8 of *fistulosum*.

The satellited chromosome of microspore 6 has an unusually long attachment thread. The second one is much larger than any measured in *cepa* or *fistulosum*. Numbers 3, 4, 5, and 6 have very similar index numbers, and it would be difficult to distinguish among 3, 5, and 6 since they all have the same length. Some are probably new types.

In microspore 7 there is a small fragment. It has the spherical shape common to nearly all the fragments found in the microspores.

TABLE 2

LENGTH AND RATIO OF SHORT TO LONG ARM IN CHROMOSOMES OF ALLIUM CEPA, A. FISTULOSUM, AND  
POST-MEIOTIC CHROMOSOMES OF THEIR HYBRID

CHROMOSOME NO.	CEPA		FISTULOSUM		HYBRID GENOMS									
	1		2		3		4		5		6		7	
	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.
1.....	38.8	0.366	30.2	0.248	41.9	0.309	27.3	0.252	33.6	0.287	33.0	0.353	27.0	0.350
2.....	29.5	0.546	39.5	0.490	37.8	0.587	34.3	0.366	29.0	0.705	47.0	0.516	37.0	0.480
3.....	31.7	0.547	29.4	0.598	25.8	0.632	31.9	0.450	41.0	0.708	37.0	0.658	31.5	0.657
4.....	31.5	0.599	31.7	0.642	41.3	0.652	31.3	0.526	23.5	0.740	33.0	0.650	37.0	0.701
5.....	42.4	0.752	36.8	0.769	27.7	0.688	29.6	0.681	38.0	0.767	37.0	0.660	34.0	0.789
6.....	38.6	0.754	29.5	0.788	43.0	0.791	42.4	0.758	43.0	0.791	37.0	0.681	47.5	0.802
7.....	28.5	0.786	32.5	0.868	34.2	0.932	37.0	0.850	33.0	1.000	28.0	0.806	36.5	0.871
8.....	18.4	0.851	31.6	1.000	26.0	0.940	35.8	0.854	25.0	1.000	27.0	0.928	44.0	0.913
9.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total lengths..	261.2	.....	259.4	.....	277.7	.....	269.6	.....	266.1	.....	279.0	.....	294.5	.....

TABLE 2—Continued

HYBRID GENOMS														
CHROMOSOME NO.	8		9		10		11		12		13		15	
	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.	LENGTH (MM.)	INDEX NO.
1.	40.5	0.500	33.0	0.375	49.0	0.400	32.5	0.585	34.0	0.545	61.5	0.366	36.5	0.303
2.	38.5	0.604	18.0	0.384	24.5	0.441	26.0	0.588	34.5	0.682	50.5	0.443	47.5	0.461
3.	35.0	0.666	5.5	0.745	21.5	0.633	27.0	0.625	17.0	0.700	33.0	0.609	35.5	0.510
4.	34.0	0.837	30.5	0.794	35.0	0.666	28.5	0.628	26.0	0.733	31.5	0.615	48.5	0.616
5.	40.0	0.860	33.5	0.914	28.5	0.676	31.0	0.631	27.5	0.833	34.0	0.780	37.5	0.875
6.	32.0	0.882	25.0	0.923	19.5	0.772	44.0	0.739	35.0	0.842	20.0	0.818	47.0	0.880
7.	23.0	0.916	43.5	0.934	31.5	0.860	25.0	0.785	21.5	0.869	32.5	0.857	28.0	1.000
8.			33.0	0.941	33.0	0.833	28.0	0.793	35.5	0.918	40.0	0.904	28.0	1.000
9.					30.0	1.000	40.0	1.000			23.0	0.916	28.0	1.000
10.											21.5	0.934		1.000
11.											40.0	1.000		1.000
Total lengths.	243.0		268.0		272.5		276.0		231.0		377.5		336.5	

Chromosomes 6, 7, and 8 all have a nearly median insertion. They are larger than the three corresponding types of *cepa* and *fistulosum* and are undoubtedly new types. There is also considerable variability in breadth among the chromosomes of this genom. Numbers 1, 2, 4, and 8 are all broader than the remaining four, 6 especially being noticeably more slender.

The situation in number 8 is interesting. All the chromosomes are shorter and thicker than in any other microspore. The absence of a satellite cannot be accepted with certainty, since it might have escaped observation in some way. It is probable, however, that a satellited chromosome was not included in the nucleus which gave rise to this group. This is the more likely explanation since there are but seven chromosomes and one fragment, and none of the seven has an index number approaching that of the satellited chromosomes of the parent species. The seventh chromosome is rather unusual; the knob on one arm is too large for a satellite and it appears to be a definite part of the main chromosome body, although it is somewhat smaller in breadth than the remainder. The same general form is also found in microspore 12. It is possible that these chromosomes may be types with secondary constrictions.

In microspore 9 the chromosomes vary considerably in size and index numbers. Numbers 5 and 8 are very similar in both length and arm ratios, but all others are easily distinguished. Number 2 is about the same size as 8 of *cepa* but is decidedly subterminal rather than median. The breadth of the proximal knob is also less than that of the distal arm. In this respect it is similar to chromosome 7 of microspore 8, and 3 of microspore 12. Number 3 is the second largest new chromosome observed in the hybrid microspores. It is exceeded only by 1 of genom 13. There is also a fragment present in this group.

There are nine rather distinct chromosomes in microspore 10. Number 1 is much larger than all the others and is subterminally constricted. It approaches the regular *Allium* satellite type, distinguished as the s chromosome by LEVAN (18). Chromosome 2 bears a satellite and has the largest index number of any satellited chromosome. In this group only 7 and 8 are difficult to distinguish.

They are nearly the same length and have somewhat similar index numbers. Number 9 may be an unmodified chromosome 8 of *A. fistulosum*.

The chromosomes of genom 11 do not contain a satellite type. Number 1 has an arm ratio of 0.585 while that of the *A. cepa* and *A. fistulosum* satellite-bearing chromosome is 0.366 and 0.248. The index number for chromosome 1 is in fact larger than the first *A. cepa* and the first and second *A. fistulosum* chromosomes. Chromosomes 2, 3, 4, and 5 would be somewhat difficult to distinguish. Number 9 with a fully median constriction is undoubtedly a new type. A fragment is present also in addition to the nine chromosomes.

The situation in microspore 12 is somewhat similar to that of 11. A satellite type chromosome is also missing. There is also more variability within the group as to length and breadth, and one less chromosome. Chromosome 3, which was mentioned earlier, is certainly a strikingly new type.

The largest new chromosome observed (number 1) occurs in microspore 13. It is about one-third larger than the longest in *A. cepa* and *A. fistulosum*, and is very much broader than any other chromosome. It is also a satellite type, having the same arm ratio as the *A. cepa* satellited chromosome. Chromosome 2 is the third largest and is also unquestionably a new type. The most unusual new chromosome observed is number 6 in this group. Each end bears a distinct satellite which differs in size in about the same degree as the satellites of *A. cepa* and *A. fistulosum*. A possible origin of this type will be discussed later in this paper. In addition to the eleven chromosomes there are two fragments.

In microspore 15, chromosome 1 is an unusual type. The main part is a satellite type chromosome and the portion attached to the proximal arm is very similar to chromosome 2 of genom 9. In this group there are also four medianly constricted chromosomes, 7, 8, 9, and 10. The first three have the same total length, but 7 is broader than the others. Number 10 is smaller, but would be difficult to distinguish from 7, 8, or 9. Since there was only one chromosome in the hybrid (number 8 of *fistulosum*) that had arms of equal length, at least three of these are new.



## FRAGMENTATION AND CHROMOSOME BRIDGES IN THE HYBRID

The frequency of fragments at each phase of meiosis and in the microspores is shown in table 3. They appear first at late pachytene and from then on to the first mitotic metaphase of the microspore. Only two were found at pachytene, one each in two cells out of 223 observed. At diplotene the cells with fragments increased sharply to 31 in 449. This percentage is not changed at diakinesis, but increases greatly at first metaphase. The frequency is again increased

TABLE 3  
FREQUENCY OF FRAGMENTS IN A. CEPA × FISTULOSUM HYBRID

STAGE IN MEIOSIS	CELLS WITH FRAGMENTS	CELLS WITHOUT FRAGMENTS	PERCENTAGE CELLS WITH FRAGMENTS
Leptotene.....	0	101	0.00
Zygotene.....	0	220	0.00
Pachytene.....	2	221	0.89
Diplotene.....	31	418	6.90
Diakinesis.....	7	99	6.60
First metaphase.....	128	521	24.56
First telophase.....	52	102	33.76
Second metaphase.....	61	200	23.37
Second telophase.....	43	134	24.29
First post-meiotic mitosis....	18	69	20.68

at first telophase, but drops again at second metaphase, and at second telophase remains about the same. In the microspores there is a further drop.

There appear to be several points at which fragments are released, the first being between pachytene and diplotene. Apparently there is no further increase until following diakinesis, but at first metaphase the frequency is quadrupled. It is further increased between this point and first telophase, at which time more cells contain fragments than at any other phase of meiosis. In the second metaphase the frequency drops again and there is no significant difference on through second telophase. The frequency at the first microspore metaphase is somewhat misleading. Unfortunately the total number of fragments in the earlier stages was not recorded at the time the data were collected. Some of these had from three to five. When the four

microspores were released, some included one or more, either by accident or because of having received them at an earlier division. It must also be remembered that these counts are from static conditions at certain phases of meiosis. The fragments are actually being released at all times following late pachytene.

The fragments found at pachytene, diplotene, and diakinesis are probably the result of the many multivalents observed during early meiosis. They also probably lack an insertion region and most likely have been formed as a result of crossing-over in possibly both homologous and non-homologous associations of terminal segments. The probable method is shown in figure 12, where two alternatives are diagrammed. In *A* and *B*, the metaphase association is shown at *m*, and the anaphase chromosomes at *a*<sub>1</sub> and *a*<sub>2</sub>. In *A*, chromosome *a-a* forms a chiasma with *b-b* at *x*. If, following either a chromatid break or formation of a new one, the realignment and orientation are as shown, there results a long chromatid, *a'-b'*, with two insertion regions, two unmodified ones, *a-a* and *b-b*, and a small fragment *b'-a'*. If, however, the realignment is as shown in *B*, there would be no chromatid bridge, but there would result, as shown in an earlier paper (9), a long + short chromatid to each pole and a small fragment. Fragments would probably be released shortly after being formed and probably account for most of those observed in this hybrid before first metaphase. Either method will account for the formation of chromosome 6 in microspore number 13.

For some reason as yet unknown, the *A* type does not seem to be common in this hybrid. Terminal associations of this sort would persist through to metaphase, and might be interpreted as polyvalents in those instances where the remaining portion of each chromosome formed chiasmata with another. Only once was such a situation observed (fig. 2*F*), although figure 2*C* is also strongly suggestive of this condition.

The sharp increase in visible fragments at first metaphase and first telophase does not mean that they have just arisen at these divisions. It is very likely they were actually formed much earlier, at the time crossing-over occurred, but were not released until these stages of meiosis had been reached. While actual chromosome breakage may occur under some conditions, the evidence is rapidly ac-

cumulating that as a rule fragments probably arise as a result of unusual crossing-over.

The decrease of fragments between first telophase and second metaphase can only be interpreted as an actual loss. Since the en-

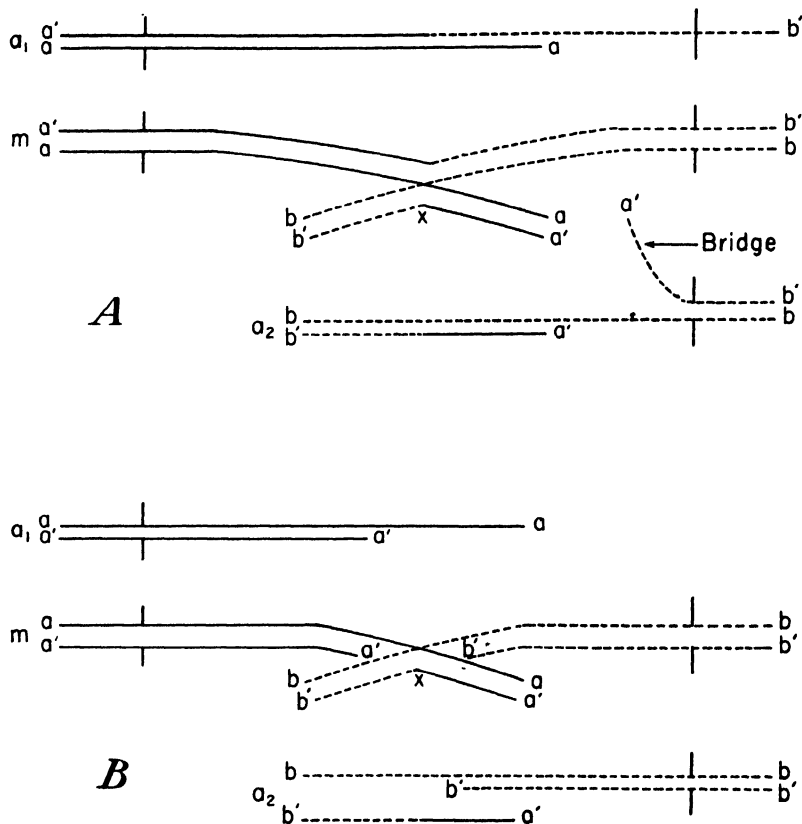


FIG. 12.—Diagrammatic details of chromatid crossing-over which results in bridge and fragment at A, and fragment but no bridge at B. Original paired chromatids shown at *m* and resulting anaphase groups at  $a_1$  and  $a_2$ . In B, realignment is not as usually accepted. (A similar diagram has been used by MÜNTZING to explain possible origin of fragments in a *Crepis* hybrid.)

tire meiotic cycle takes place within the pollen mother cell wall there is no opportunity for any fragments to be cast out of the cell. In figure 5B, C, and E the fragments appear to be disintegrating. LEVAN (19) also reports the same situation in his *A. cepa* × *A.*

*fistulosum* hybrid, and similar observations have been reported by other investigators in other genera.

It is now generally accepted that the survival of a fragment depends on its possessing an insertion, and to a lesser degree a length sufficient to assure chiasmata formation with its homologue. Most of the fragments in this hybrid probably lack an insertion. Only a very few were observed to divide, and certainly all those resulting from formation of chromatid bridges have no insertion. While some are included in the microspores, none have been found in any of the twenty-five backcross plants which originated from pollen of the hybrid used on *A. fistulosum* and *A. cepa*. The fragments in this hybrid either persist until lost as micro-nuclei when the spore mother cell wall breaks down, or they disintegrate in the cytoplasm at earlier stages.

The atrophy and loss of some fragments, while others apparently persist, is of considerable interest. Just what happens to cause disintegration is not known. Possibly they are digested by enzymes in the cytoplasm, if they are unable to form a protective interface or chromosome membrane. In the resting cell the nuclear membrane may be considered as such an interface, which protects the chromosomes while they are undergoing metabolic changes prior to the next division. When the chromosomes contract, each appears to have formed its own membrane or interface by late prophase when the nuclear membrane disappears. The chromosomes at this time are in direct contact with the cytoplasm, and are perhaps analogous to nuclei. When a piece of chromosome is unable to form a protecting interfacial membrane it is very probably attacked and destroyed. The loss of such pieces includes, of course, the genes they carry. If the deletion is very small, the deficient chromosome may function and be transmitted to the next generation. Eventually there may arise individuals, heterozygous or homozygous for the deficiency, which because of it possess new or modified characters.

The formation of so-called chromosome bridges has now been observed cytologically in a wide variety of material. Some of these are listed in table 4. From this table it can be seen that the situation has been found in untreated as well as treated plants. Undoubtedly many earlier observations which were interpreted as non-disjunc-

TABLE 4  
OCCURRENCE OF CHROMATID BRIDGES IN PLANTS

GENUS	MEIOSIS	MITOSIS	INVESTIGATOR
Untreated species			
1. Rumex.....	I A	.....	Meurman, 1925 (29)
2. Ribes (3 species).....	I A	.....	Meurman, 1928 (30)
3. Zea (sticky gene).....	I A, II A	.....	Headle, 1932 (2)
4. Paronia (4 species).....	I A	.....	Hicks and Stebbins, 1934 (13)
5. Matthiola (crenate).....	I A	.....	Armstrong and Huskins, 1934 (1)
6. Trillium.....	I A, II T	.....	Smith, 1935 (38)
7. Secale.....	I A	.....	Lamm, 1936 (17)
8. Paeonia.....	I A	.....	Dark, 1936 (5)
9. Aesculus.....	I A	.....	Upcott, 1936 (39)
10. Tulipa.....	I A	.....	Woods and Bamford, 1937 (40)
11. Campanula.....	I A	.....	Darlington and Gairdner, 1937 (6)
12. Tradescantia (triploid).....	I A, II A	Microspore	Sax, 1937 (37)
13. Lilium.....	.....	T	Emsweller and Brierley (unpublished)
14. Narcissus.....	.....	A	Emsweller (unpublished)
15. Allium (cepa).....	I A	.....	Emsweller and Jones (this paper)
16. Lactuca.....	I A	.....	Whitaker and Jagger, (in press)
Irradiated stocks			
1. Tulipa.....	I A	.....	de Mol, 1930 (7)
2. Zea.....	I A	.....	McIntock, 1931 (28)
3. Nicotiana.....	I A, II A	.....	Goodspeed and Avery, 1933 (11)
4. Crocus.....	.....	A	Mather and Stone, 1933 (27)
5. Tulipa.....	.....	A	Mather and Stone, 1933 (27)
6. Vicia and Tradescantia.....	I A, II A	.....	Mather, 1934 (25)
7. Allium.....	I A, I T	.....	Levan, 1936 (22)
Species hybrids			
1. Godetia.....	I A, I inter, II A	.....	Chittenden, 1928 (4)
2. Aegilops.....	I A, II T	.....	Miczynski, 1931 (31)
3. Nicotiana.....	I A	.....	Müntzing, 1934 (32)
4. Crepis.....	I A, II A	.....	Müntzing, 1934 (32)
5. Hemizonia.....	I A	.....	Clausen, 1934 (32)
6. Allium.....	I A	.....	Levan, 1935 (19)
7. Triticum.....	I A	.....	Mather, 1935 (26)
8. Pisum.....	I A, II A, II M	.....	Hakansson, 1936 (12)
9. Lilium.....	I A, II A	.....	Richardson, 1936 (36)
10. Lilium.....	I A, II A	.....	Ribbans, 1937 (35)
Generic hybrids			
1. Aegilops × Triticum.....	I A	.....	Longley and Sando, 1930 (23)
2. Fuchsia × Zea.....	I A, I T	.....	Beadle, 1932 (2)
3. Triticum × Secale.....	.....	I T	Plotnikowa, 1932 (34)
4. Triticum × Haynaldia.....	I A	.....	Kihara and Nishiyama, 1937 (15)
Exposed to low temperature			
1. Rheo discolor.....	.....	Microspore division	Dermen (unpublished)

tion or lagging univalents also belong in this list. It is interesting to note that bridges have been found in both meiosis and mitosis, in the latter also occurring both spontaneously and as a result of x-radiation.

The formation of such doubly inserted chromatids in meiosis has generally been explained as the result of crossing-over in inverted sectors. There is, however, some evidence in this hybrid that bridges also arise when inversions are probably not concerned. When two chromosomes pair in such a manner that their insertion regions are not opposite each other, and a single cross-over occurs in the interval between the insertions, a bridge will ordinarily result. As pointed out earlier, in the *cepa* × *fistulosum* hybrid such type of pairing appears to be common. In the two species, the relative location of homologous blocks of genes in two synapsed chromosomes probably causes such an association. The situation is probably brought about, therefore, more as a positional effect of homologous sectors rather than simple inversions. The single bridge and fragment observed at the first division in the *A. cepa* parent of this hybrid cannot be explained on the basis of crossing-over in inverted sectors, unless the inversion arose in a pre-meiotic mitosis. In like manner the bridges observed in mitosis as a result of x-radiation, and spontaneously in *Narcissus*, while probably arising from some sort of crossing-over, are probably not due to inverted sectors. It seems more likely that bridges arise in a number of ways from unusual crossing-over. Some of these causes may be translocations, or simple rearrangements of insertions as well as inversions.

In this material, there is also evidence that bridges do not actually break as a result of tension in the chromatid. It has become general to speak of chromosome threads, tensions, and torsions as if we were dealing with a mechanical structure. If this is the case, the chromatid forming a bridge should, following the break, move somewhat rapidly to the daughter nuclei. The fact that they do not do so is shown by frequent observations of free ends jutting into the cytoplasm after such a supposed break has occurred. In many instances these free ends also exhibit various degrees of disintegration similar to that observed in fragments. In addition, the bridges commonly show two "breaks" with an isolated fragment left out in the cyto-

plasm between the free ends of the chromatid. This has also been observed in *Lilium longiflorum* by EMSWELLER and BRIERLEY (unpublished). If the chromatid does break under tension, it is inconceivable that two simultaneous breaks could occur in such a short distance. The writers are inclined to the opinion that the same activity is probably responsible here as in the case of disintegration and loss of small fragments. When a chromatid with two insertion regions is pulled out across the cell, it finally becomes very attenuate. The resulting bridge usually persists unbroken through anaphase and usually well into telophase. At this time the daughter nuclei are being organized, the chromosomes losing their visible identity, and the formation of nuclear membranes just beginning. The attenuated chromatid is now left out in the cytoplasm where it probably attempts to go through stages comparable with the portions included in the daughter nuclei. It seems likely also that its membrane is breaking down, and it becomes highly susceptible to attack from forces in the cytoplasm. In such a way the chromatid may be destroyed at one or more places, giving rise to the "double breaks" as shown in figure 6*B* and *D*.

The occurrence of bridges in the second division of meiosis has been explained as a result of their persistence after failing to "break" at the first division. In our hybrid this probably does occur occasionally (fig. 7*D*), but it is not common and certainly cannot explain the high frequency of second division bridges. In the hybrid, as well as *A. cepa* and *A. fistulosum*, cytokinesis follows rapidly after the first division. By second metaphase the cytoplasm is well organized in two distinct bodies which may easily be separated by rupturing the pollen mother cell wall. When bridges do occasionally persist into the second division, it is probable that such cells become disorganized, as is apparently the situation in figure 7*D*. Since the evidence indicates that only a very few first division bridges persist into the second, the majority must reach this stage in some other way. As pointed out earlier, when two paired chromosomes with their insertions not opposite cross over in the region between them, there results a chromatid with two points of attachment. Similar unusual chromatids are also formed in other ways, as already noted. Since the insertions are not exactly opposite, they probably segre-

gate to the poles independently, so that both go to the same one about 50 per cent of the time, and to opposite ones with like frequency.

However, bridges of the "loop" type observed by SMITH (38) in *Trillium* always go to the same pole in the first division and then form a bridge at the second. He also found a second type which always formed a bridge at first division. The loop bridge was the result of a double cross-over, one within the inverted region and one proximal to it. The second bridge resulted from a single cross-over in the inverted sector. In both types the insertions were exactly opposite each other and segregated in the normal manner, one to

TABLE 5  
FREQUENCY OF BRIDGES AT FIRST AND SECOND MEIOSIS

FIRST DIVISION			SECOND DIVISION		
NO. CELLS OBSERVED	NO. BRIDGES	AVERAGE PER CELL	NO. CELLS OBSERVED	NO. BRIDGES	AVERAGE PER CELL
858	148	0.1724	229	32	0.1397

each pole. The second type was the most common and when present would show an unexpected increase in first division bridges.

A count of the relative frequency of bridges in first and second divisions in the *cepa* × *fistulosum* hybrid is shown in table 5.

If all the bridges formed in this hybrid were of the type that permitted independent segregation of the two insertion regions, we might expect to find about as many at the second as at the first division. Here, however, it must be pointed out that some of the second division bridges should be so oriented that they again go to one pole and become included in the microspore nucleus. On a purely chance basis then, there should be about half as many bridges at second as at first division. It is probable that some of the types formed in this hybrid do not appear until the second division. This would account for the unexpectedly high frequency of bridges at second division.

A comparison between the normal and one method of bridge



formation in meiosis and the first pollen grain division is shown in figure 13. If a cross-over occurs between the attachments, a bridge will be formed provided the insertion regions are oriented to opposite poles. If they go to the same pole a bridge will not be formed, but in each type a fragment will occur. In the second division the bridge chromatid in a nucleus behaves essentially the same as at the first division. If the two insertion regions of the same chromatid are oriented to different poles, a bridge results; if not, they go to the same pole and enter a microspore nucleus. The second division merely completes the separation of chromatids held together at the attachments, and there is no splitting as occurs in mitosis. Thus a second division bridge is a single structure, as shown in figure 7C. In the microspore the division is a normal mitosis and the chromosome with two insertion regions is split longitudinally. As pointed out by MATHER and STONE (27), the mitotic division of such a chromosome may result in perfect separation, or in the formation of two bridges which usually will lie across one another forming an X configuration as shown in II of figure 13. If the chromosome is oriented as at I in figure 13, there will be no bridge. If the chromatids become twisted, however, so that the insertions on each one are oriented to opposite poles, the two bridges will arise.

We did not observe any bridges in microspore divisions, but this cannot be accepted as final evidence since so few microspores in the hybrid are normal, and bridges can be identified with certainty only at anaphase and telophase. We are deeply grateful to Dr. ALBERT LEVAN, who while at the United States Horticultural Station, Beltsville, Maryland, kindly allowed us to examine some of his slides of irradiated *Allium schoenoprasum*. In this material we did find a bridge in the anaphase of the first division of a microspore. Since this material had been irradiated during early prophase, this bridge was probably formed sometime during early meiosis. Double attachment chromosomes at both metaphase and anaphase of pollen grain divisions in irradiated *Tradescantia* have been reported by MATHER (25). SAX (37) also found bridges in microspores of a triploid *T. bracteata*.

Just as this paper was completed we were fortunate in finding cytological confirmation for the figure of a microspore bridge as

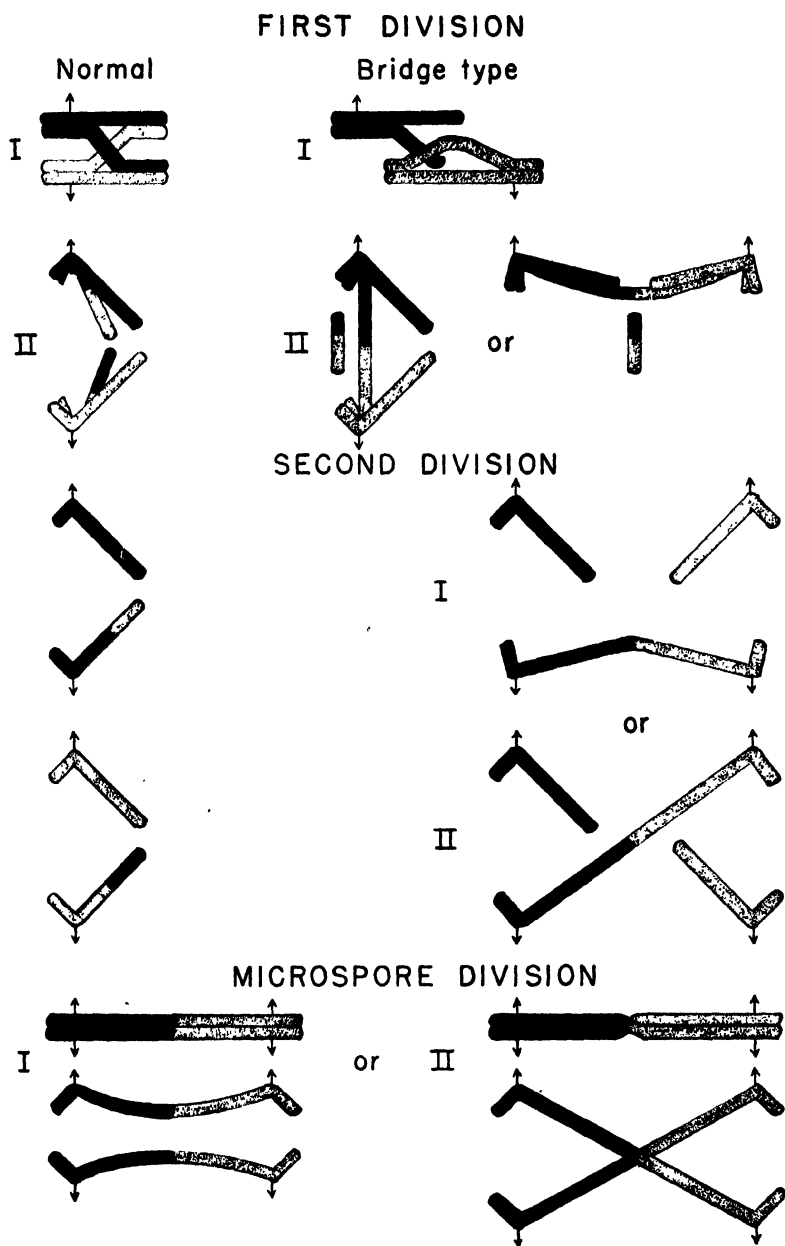


FIG. 13.—Diagram showing origin of chromatid bridges in meiosis, and their behavior through first microspore division.

shown in the diagrammatic drawing of figure 13. The photomicrograph is shown in figure 14. It is from a dividing microspore of *Rhoeo discolor* taken from a plant that had received a low temperature exposure. The plant was grown in a greenhouse at 60° to 70° F. and moved to a low temperature of 40° to 50° overnight. This slide



FIG. 14.—Dividing microspore of *Rhoeo discolor* from plant exposed to low temperature during meiosis. Note X configuration of two bridges.

is from material collected 11 days following exposure. We are grateful to Dr. HAIG DERMEN of this Division, who kindly permitted us to use this figure. This bridge is a cytological demonstration of the second type of microspore division as shown in figure 13.

If a chromosome with two insertions is included in a functional

microspore, it may eventually reach a zygote where it could conceivably persist for several cell generations. The bridges observed by PLOTNIKOWA (34) in derivatives of his wheat-rye hybrids were probably the result of somatic crossing-over, since single bridges and not X configurations were observed in each instance. In addition a mitotic chromosome with two insertions, even though forming two bridges, would not be accompanied by a fragment such as shown in his figures. The bridge observed by EMSWELLER in a *Narcissus* root tip was also single and was accompanied by a fragment. This was also true of those observed by EMSWELLER and BRIERLEY in somatic divisions of *Lilium*.

### Discussion

The high frequency of bivalent formation in this hybrid is undoubtedly indicative of the presence of many common genes in the two species. It also indicates that crossing-over is frequent, and this is further borne out by the new chromosome types found in the first post-meiotic mitosis. Since all the hybrid microspores examined contained one or more new chromosomes, it seems very likely that some of these new forms have been transmitted to the backcross plants now in our cultures.

The formation of these new chromosomes is undoubtedly the result of the unusual crossing-over described earlier in this paper, which produced translocations, deletions, and fragmentations, and is probably responsible for the observed changes in chromosome morphology. There are also probably many other changes too small to be seen cytologically, but possible of causing new pairing conditions in subsequent generations. In addition, the formation of these new chromosomes indicates that chromosome individuality is dependent on the existence of normal pairing such as occurs in a well established balanced species. When this balance is upset, the chromosome is found to be plastic and capable of considerable change in its morphology. These changes are certain to produce irregularities in the next generation, however, and probably only very minor ones are transmitted. Thus the maintenance of chromosome morphology in a species is probably protected by the inability of structurally changed chromosomes to survive in competition with normal ones.

All of the fragments found in this hybrid are most reasonably explained as products of crossing-over. They also all appear to be a piece of but one chromatid. Under some conditions they are freed from the remainder of the chromatid as early as late pachytene, but are most frequent at first telophase. Fragments as early as pachytene have not heretofore been reported. Their appearance thus early indicates that the mechanics of crossing-over is completed at an early stage of meiosis.

The behavior of chromatid bridges at first and second division of the hybrid is very clear. In the first, each bridge is composed of three chromatids, one having two insertions and the others one each. They are ordinarily accompanied by a fragment, although it may not be present if it was released earlier and was not able to survive thus far. DARLINGTON and GAIRDNER (6) also report bridges without fragments in *Campanula persicifolia*, and suggest that some of the fragments are too small to be seen.

The bridges in this hybrid probably do not break but are destroyed by some cytoplasmic activity, possibly enzymatic. When this occurs, the parts left out in the cytoplasm appear to disintegrate rather than to be drawn in and incorporated in the new forming nuclei. This situation was inferred from the actually observed breakdown of bridge remnants still jutting into the cytoplasm during late anaphase, telophase, and even at interkinesis (figs. 5*B*, *D*, *E*, *F*; 6*A*, *B*, *C*, *D*; and 9*B*, *C*). There are also instances of more than one point of disintegration in the same bridge (fig. 6*B*, *D*).

The bridges of the second division are composed of a single chromatid with two insertion regions. They occur at the second chromosome division cycle following the cross-over which produced them. None of these bridges could possibly persist into a microspore, and none were ever found in well developed tetrads. That bridges do reach microspores has been demonstrated, however, and SAX (37) has explained their presence in triploid *Tradescantia bracteata* as being derived from dicentric chromatids broken at meiosis and re-joining at the first post-meiotic division.

Disintegration in the cytoplasm of parts of chromatids is of theoretical and probably of practical interest. The genes carried by these

bits of chromatin are lost, and the resulting deficiency may produce a profound change in the tissue descending from such a deficient cell. It seems likely that minute losses of this sort are more common than has previously been supposed. It is probable that more and more of them will be reported, since the bridge and fragment mechanism is now more clearly understood. In addition, there are probably other ways in which small fragments may arise and be lost in both meiosis and mitosis. It is not to be inferred that all spontaneous bud sports or mutations arise in this way, since reverse mutations eliminate a loss theory. It is probable that a number of causes are active, but it appears likely that spontaneous deficiencies may explain some of the many irreversible bud sports that occur with such comparatively high frequency on many horticultural plants.

### Summary

1. The hybrid between *A. cepa* and *A. fistulosum* is unusual because of the high frequency of complete pairing, fragmentation, and formation of chromatin bridges.

2. Fragments were found as early as late pachytene, increasing in frequency to first telophase, then dropping. Their origin is explained as a result of unusual crossing-over. The decrease in number is probably due to an actual loss. Their possible digestion in the cytoplasm by enzymes is suggested as an explanation for this.

3. Chromatin bridges were found at first and second anaphases and telophases. It is suggested that these bridges probably arise from unusual crossing-over, some in inverted sectors, but to a considerable extent because of positional effect of insertion regions or homologous sectors. The cytological evidence also points to some interpretation other than breakage as an explanation of their dissolution. From the behavior of these bridges it was inferred that some reach the microspores, depending on the chance orientation of their two insertion regions. In the microspore mitosis they will form an X configuration when the insertion regions of one chromatid are oriented to opposite poles. This was confirmed cytologically on a slide from *Rhoeo discolor* kindly lent us by Dr. DERMEN of this Division.

4. The presence of morphologically new chromosomes was determined in the first division of hybrid microspores. These were undoubtedly formed by crossing-over between the two genomes.

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# SYNTHESIS OF FATS BY GREEN PLANTS<sup>1</sup>

GEORGE O. BURR AND ELMER S. MILLER

(WITH THREE FIGURES)

## Literature review

In 1804, DE SAUSSURE (22) studied the respiratory quotients of many species (*Quercus*, *Aesculus*, *Robinia*, *Sedum*, etc.) and reported that the value of the quotients was unity. He also found a respiratory quotient of 1.0 for germinating seeds (*Vicia faba*, etc.). In 1849, REGNAULT and REISET published their experiments which showed that the value of the respiratory quotient depended on the nature of the food given to animals. By 1866 PETTENKOFER and VOIT had developed the method of measuring change in weight, respired water, and carbon dioxide, and from these figures calculating what substances had burned in the body.

BERT (2) seems to have been the first physiologist interested in plants to study the respiratory quotient under different circumstances and to discuss the results in relation to formation and destruction of reserve food. DEHÉRAIN and MOISSAN (5) concluded that the evolution of carbon dioxide and absorption of oxygen in respiration were not directly related, a view not accepted by BONNIER and MANGIN.

Although both PRESTA and DE LUCA (14) showed that olives could make oil after separation from the tree, GODLEWSKI (10) for the first time measured the respiratory quotient of ripening oily fruits. Using excised seeds of *Papaver* and *Ricinus*, he found values of 1.18 to 1.52. He discussed the effect on the respiratory quotient of chemical composition, fermentation, and change of fat to starch. BONNIER and MANGIN (3), in their series of papers published from 1884 to 1886, improved methods of analysis and studied the effect on respiratory quotient of temperature, oxygen tension, light, anesthetics, developmental periods, season, etc. Their studies were made on fungi, germinating seeds, and green leaves of many species. With the last they attempted separation of respiratory and photo-

<sup>1</sup> A contribution from the Department of Botany, University of Minnesota.

synthetic quotients. Although they recognized that the respiratory quotient was not a constant, they made no effort to use it as a measure of the nature of the metabolic process in progress.

In the same years that ATWATER and coworkers in this country and ZUNTZ and coworkers in Germany were determining exactly the respiratory quotient and calory value of animal foods, GERBER (7, 8, 9) in France was conducting extensive experiments in an effort to determine the nature of the metabolic process from the respiratory quotient, supplemented by tissue analysis. He followed the

TABLE 1  
RESPIRATORY QUOTIENTS OF OLIVE SEEDS  
DURING MATURATION

DATE	COLOR OF SEED	RESPIRATORY QUOTIENT
July 15.....	Green olive	0.79
October 6.....	Color changing	1.51
October 22.....	Ripe olive	0.68

gas exchange of detached fruits and seeds inclosed in a respiration chamber in order to determine whether the fat was formed in the leaves and then translocated into the fruit or whether the fat was formed in the fruit from carbohydrates. His first work was with olives. It had been shown by DE LUCA (14) that green olives were very rich in mannite, which was replaced by oil as the olives ripened. GERBER's respiratory quotients of olive seeds (during maturation) are summarized in table 1.

GERBER made a similar study with *Ricinus* and described the results as follows: The respiratory quotient of *Ricinus* seeds is less than unity during the very early age while they are soft. During this period the amount of glucose and sucrose is considerable, while oil is absent or present only in traces. The respiratory quotient rises above unity when the seeds have become somewhat firm. During this period the proportion of sugars decreases and the oil increases. When the seeds are hard and fully ripe, sugars are present only in traces and oil has reached a maximum value. The respiratory quotient has now fallen below unity.

GERBER concludes that his results confirm the theory of MUNTZ and of DU SABLON that fats are formed in fruits and seeds from carbohydrates.

In his later work GERBER divides the ripening of olives into four phases:

1. Transformation of mannite to oil, 4-5 days long. Respiratory quotient falls from 1.45 to 1.14.

2. Complete oxidation of a little oil, 15-30 days long. Respiratory quotient falls from unity to about 0.71.

3. Transformation of a little of the oil into carbohydrate. A short period in which respiratory quotient is 0.55-0.65.

4. Complete oxidation of carbohydrate formed in preceding period. Respiratory quotient rises to as high as 0.88 and then falls to 0.75.

From a study of temperature coefficients and surface effects, GERBER concludes that the respiratory quotient of oil formation can be distinguished from the respiratory quotient of either acid burning or ordinary fermentation.

McCLENAHAN (16, 17) analyzed black walnuts at 14-day intervals during the period of rapid development. Since no starch, sugar, or tannin was detectable in the kernel at any period, he concluded that fat in the walnut is not formed from these substances and considered the possibility that fatty acids moved into the capsule. However, the evidence seems inadequate for his suggestion that fat is formed from tannin in the hull. He also reports a large percentage of water soluble material in the alcohol-ether extract during the period of rapid development and oil formation. Some of this might be a mobile lipid, but the method of extraction gives no real clue as to the nature of this material and he presents no further chemical evidence.

McCLENAHAN made the interesting observation that the xylem was very rich in stainable fat while the phloem contained none. This might have some bearing on fat transport, although it is believed by some workers that concentration of a compound in xylem or phloem does not mean that tissue is the path of transport.

Fat formation has been studied more extensively in flax than in any other seed. IVANOV (12, 13) observed that during the period of

fat synthesis there was a decrease in the amount of total carbohydrate. He also concluded that saturated fatty acids were formed first and these in turn were desaturated. Later work with flax has brought out the following points. The seed develops to full size before rapid oil formation begins (6). Then in a period of about fifteen days all of the oil is laid down. The percentage of oil in the dried seed reaches a maximum within twenty-four days after flowering, but desaturation continues until full maturity (EYRE, quoted by ARMSTRONG and ALLAN 1). Lipase activity of flax seed decreases rapidly during the period of oil formation (23). The most marked decrease in enzymatic activity takes place just after the period of most rapid oil formation, the seventeenth day (24). There is no low molecular weight intermediate that accumulates to a measurable degree, since the saponification value and neutralization number of the oil are constant throughout the development of the seed.

### Investigation

This paper presents a study of the value of the respiratory quotients of the seeds of castor bean, *Ricinus communis* L. var. *lividus* (Jacq.) (*R. sanguineus* Hort., *R. obermannii* Hort.), during seed development and maturation. It was our purpose to secure more complete data on gas exchange of oily seeds measured by the most accurate methods now in use. The seeds are kept attached to the plant so that normal exchange of solutes between the plant and seed will continue during the experiment.

MATERIAL.—For a study of this type, the plants should fulfil the following requirements: (a) The fruit must synthesize and store a considerable amount of fat. (b) The fruits should be capable of developing and maturing under greenhouse conditions. (c) The seeds should, preferably, be borne separately. Such an arrangement permits the removal of one or more seeds for fat analyses without materially disturbing the others. (d) The fruits and leaves should be so arranged that they may be conveniently isolated in a plant chamber. (e) The plant should be so sturdy as to permit repeated handling. These requirements were met satisfactorily by potted castor bean plants.

**APPARATUS.**—The apparatus employed is illustrated in figure 1. *C* is a constant speed spirometer which forces a gas stream of known composition through the metabolism chamber. *F* is a series of collecting bulbs which continuously sample the outgoing gas stream. They are full of dry mercury at the beginning of a run and are so arranged that only one is collecting gas at a time. The rate of collection of gas is controlled by the windlass which is on the same

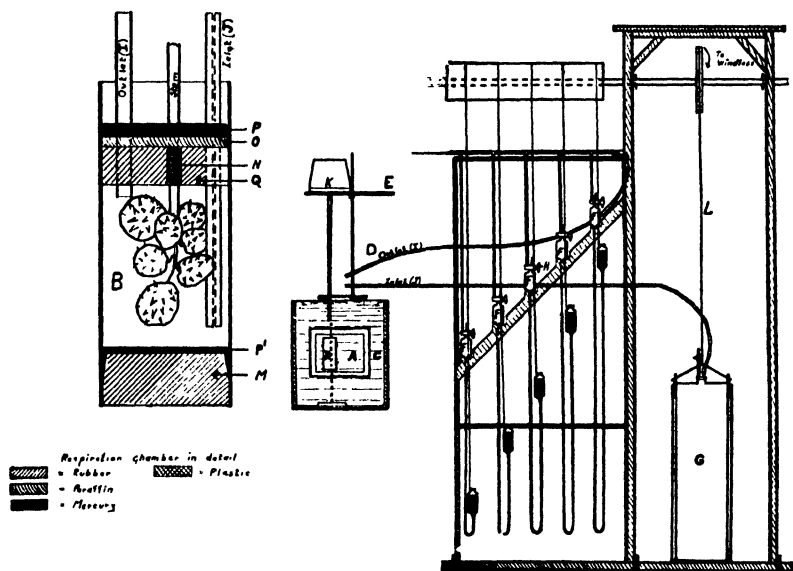


FIG. 1.—Apparatus for continuous sampling of gas stream; detailed drawing of respiration chamber at left.

shaft as the spirometer. Thus a constant fraction of the total gas stream is always being taken. The total number of samples and the period covered by each sample can be altered at will. By the insertion of capillary mercury traps above each sampler, the system becomes entirely automatic. If the air stream is dried after leaving the metabolism chamber, samples may be held without change until it is convenient to make the analyses.

A HALDANE-CARPENTER (4) apparatus was employed for the quantitative determinations of oxygen and carbon dioxide. The analyses were made in the manner described by MILLER and BURR

(19). The respiration chamber and the seal employed in this study are illustrated in detail in figure 1. The dimensions of chamber *B* are: diameter 57 mm., length 150 mm. The lower end is closed by a rubber stopper, *M*, which is covered by a layer of mercury, *P*. About 40 mm. from the other end, a split stopper, *Q*, is inserted. The space between the stopper and seed stalk is filled with a plastic, *N*. A layer of low melting paraffin is poured above the stopper, care being taken that the temperature of the paraffin does not exceed 45° C. After the paraffin has solidified, it is covered by a layer of mercury, *F*. This type of seal does not injure the seed stalk. As long as a layer of mercury remained at each end of the chamber, no leaks could be detected. The distance that the cut stopper is inserted into chamber *B* depends on the size of the cluster of castor bean seeds. In all the experiments the volume of chamber *B* is kept as small as possible, 150 cc. or less. The diameter of the inlet tube, *J*, is 1 mm. and of the outlet tube, *I*, 6 mm. The chamber is immersed in a water bath which is maintained at a temperature of 28°–29° C. by a heater and thermostat.

#### DIFFUSION OF CARBON DIOXIDE AND OXYGEN THROUGH CASTOR BEAN PETIOLES

Before the study of the gaseous exchange of different organs was undertaken, the rate of diffusion of carbon dioxide and oxygen through castor bean petioles was investigated. In all the following experiments, the seal illustrated in figure 1 was employed.

In experiments 1 to 4, the plants were brought into the laboratory twelve hours before the diffusion experiments were begun. In these experiments the leaves were inclosed in 3-liter Erlenmeyer flasks. As an aliquot portion of gas was removed, an equal amount of water was introduced into the respective flasks in order to maintain the same pressure around the inclosed leaves as that surrounding the rest of the plant.

Before carbon dioxide was introduced into the flasks, the rate of respiration was determined over a 2-hour period. Carbon dioxide was introduced into the leaf chamber and analyses were made at the beginning and at the end of a 2-hour period. After the carbon dioxide had been replaced by air, the rate of respiration was again

determined for another 2-hour period. From the average rate of respiration for these two periods, the rate of respiration was calculated for the period when the leaf was in an atmosphere containing approximately 1 per cent carbon dioxide.

The data presented in table 2, experiment 2, may be used to illustrate the calculations of the values in the columns under "calculated" and "observed." A leaf was inclosed in the respiration chamber containing room air (0.036 per cent carbon dioxide), and the rate of respiration was determined for three hours. During this period the leaf respired 7.027 mg., or 2.342 mg. per hour. Carbon dioxide

TABLE 2

## DIFFUSION OF CARBON DIOXIDE THROUGH CASTOR BEAN PETIOLES

EXPERIMENT	DURATION (HOURS)	RESPIRATION PER HOUR (MG.)	OBSERVED MG. CARBON DIOXIDE	CALCULATED MG. CARBON DIOXIDE	PERCENTAGE DIFFERENCE
1.....	1 33	0.19	53.40	53.60	-0 4
2.....	3.00	2.42	61.21	61.18	+0 05
3.....	2.92	1.19	53.15	53 10	+0.1
4.....	3.83	2.96	11.63	11.44	+1 7

was introduced into the respiration chamber, and the analyses of the aliquot portion showed that the atmosphere contained 53.924 mg. Three hours later the atmosphere contained 61.218 mg. The respiration chamber was now opened and permitted to come to equilibrium with room air. The rate of respiration was again measured and found to have increased the carbon dioxide concentration of the atmosphere by 7.495 mg., or 2.498 mg. per hour. The average of the rate of respiration of the first and last period was employed in calculating the amount of respiration which occurred during the period that the leaf was in an atmosphere enriched with carbon dioxide. This correction was 7.261 mg., which must be added to 53.924 mg. Hence the calculated value was 61.185 mg., as shown in the table. It was assumed that the higher concentrations of carbon dioxide had no effect on the rate of respiration.

In view of the fact that a different plant was used in each of the preceding experiments, the results indicated in table 2 are in excellent agreement. The error recorded is approximately equal to the



total possible analytical error of the three carbon dioxide determinations by the HALDANE-CARPENTER apparatus. It would appear, therefore, that with a carbon dioxide concentration difference of about 1 per cent, no appreciable loss takes place by diffusion through the petiole.

A few experiments were made to determine the rate of diffusion of oxygen through castor bean petioles. The data summarized in table 3 were obtained by the same type of calculations as that described for carbon dioxide.

The rate of oxygen consumption by leaf respiration was determined for an initial period. Then the atmosphere in the respiration

TABLE 3  
DIFFUSION OF OXYGEN THROUGH CASTOR BEAN PETIOLES

EXPERIMENT	DURATION (HOURS)	RESPIRATION PER HOUR (MG.)	OBSERVED MG. OXYGEN	CALCULATED MG. OXYGEN	PERCENTAGE DIFFERENCE
21.....	2 0	0.888	21.40	20.30	+5.1
22.....	2.0	1.172	16.06	16.36	-1.8
23.....	2.0	1.134	13.92	13.72	+1.4
24.....	3.0	0.888	19.86	18.74	+5.6

chamber was enriched in oxygen by approximately the amount recorded in the column under "observed." In these experiments it was necessary to assume that increasing the oxygen content of the atmosphere by approximately 0.5 per cent had no effect on the rate of respiration.

The maximum analytical error for oxygen determination ( $\pm 0.005$  per cent) is so great that in a series of analyses on such small samples, the summed error may become 5-10 per cent of the total respiration; therefore these figures do not have great exactness. The differences found are within the experimental error, and since these are usually positive, there is a strong indication that with a 0.5 per cent gradient, oxygen does not diffuse at an appreciable rate through leaf petioles.

#### MEASUREMENT OF RESPIRATORY QUOTIENTS OF SEEDS

After the plant had remained one hour in the laboratory, the seeds were inclosed in the respiration chamber. The apparatus was run for

two hours before the collection of samples was begun. A sample was collected every five or six hours during each run.

In this study, the respiratory quotients were determined at 6 to 9-day intervals after fertilization of the ovule. Following each experiment, one seed ball was removed from the plant for quantitative analysis of the total fat content. The seeds were dried in a vacuum oven at 40° C. to a constant weight, and then stored in an atmosphere of carbon dioxide until analyzed. The A.O.A.C. method (20) was employed for determination of the total fat content.

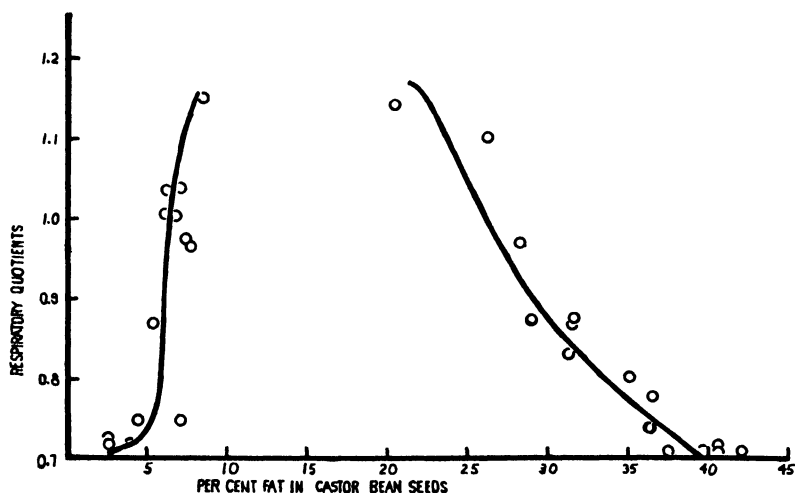


FIG. 2.—Respiratory quotient of castor beans plotted against oil content. Most of the oil appears during a few days of high respiratory quotient.

The results of these determinations are presented in figures 2 and 3. The first measurements (shown by the circles in the lower left hand corner) were made approximately seven days after fertilization. During this early period, the quotients remained below 0.75 and the total fat was between 3 and 6 per cent of the dry weight. During the next fifteen days there was a gradual increase in respiratory quotient with very little increase in fat. It was only after the respiratory quotient had risen above 1.00 that rapid accumulation of fat occurred. However, there was an appreciable increase in fat after the respiratory quotient had again fallen below 1.00 and even below 0.8.

These data confirm the observations of GERBER (7, 8, 9) and GODLEWSKI (10) that the respiratory quotient is greater than 1.00 during the period when fat accumulation is at a maximum. These high quotients are in accord with the view that considerable fat synthesis occurs in the seeds from sugars brought in from the leaves. They do not exclude the possibility, however, that some fat is synthesized in other tissues and then transported to the seeds.

In fact, a slow accumulation of fat takes place during the very early period and again during the final ripening period when the

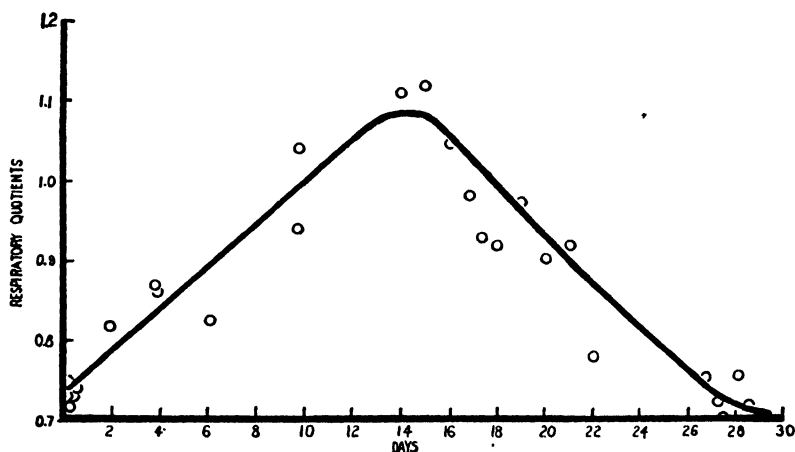


FIG. 3.—Respiratory quotient of castor beans at various stages of maturity

quotients are well below unity. These data indicate an inward movement of lipid. However, such an interpretation loses much of its argument in view of the fact that GERBER found low quotients with detached fruits.

That the leaf is capable of furnishing a ready supply of lipid material is indicated by the extensive work of MAQUENNE and DEMOUSSY (15). Using improved methods, they found high and fairly uniform values for respiratory quotients of all leaves. An average value of 1.046 is given for hundreds of determinations on forty species whose leaves were fresh and active (15, p. 111). Such a value indicates that the average leaf is actively producing fatty acids, alcohols, hydrocarbons, or other compounds containing little

oxygen as long as it is well supplied with carbohydrates. Some of this lipid material might be translocated. A young active leaf, when kept at high temperatures, may so very quickly deplete its store of carbohydrates that after a few hours in darkness its quotient has fallen to 0.8–0.9 (15, pp. 42–43).

A respiratory quotient of 0.7 indicates a use of pure fatty acids or some hydrocarbons or alcohols as fuel for respiration. However, since fat is slowly accumulating, its use in respiration would require that even larger amounts be moved in from the rest of the plant. On the other hand, if carbohydrates chiefly are being burned, this process must be accompanied by the synthesis of compounds of still higher oxygen content. Acids such as malic, tartaric, oxalic, citric, glucuronic, and galacturonic are richer in oxygen than glucose, and their formation could produce quotients as low as 0.7 provided several molecules of acid were formed for each molecule of glucose burned to carbon dioxide and water. For example, if glucuronic acid were being formed for the production of gums and hemicelluloses, three molecules of this acid would be required for each molecule of glucose burned to give a quotient of 0.67. This quotient is low enough to mask slow fat synthesis when the observed (composite) respiratory quotient is between 0.7 and 0.8.

RHINE (21) was confronted with a similar difficulty in interpretation of respiratory quotients. Working with both oily and starchy seeds he found the respiration quotient of the hypocotyl to be about 0.77. This might indicate that fats were being transported to the growing tip and burned. However, by analysis he found a concentration gradient in the opposite direction and concluded that the movement of oils as such is improbable. It is well to remember that gradients calculated from total ether extract of tissues are under suspicion since a large portion of total lipids in active cells may be combined and non-mobile. MILLER (18) found that during the last week of germination the free acids were nearly all of low molecular weight and soluble in water. These fatty acids are mobile, undetectable by staining methods, give low respiratory quotients when burned, and could be made into higher fatty acids with little effect on gas exchange ratios.

A paper by GUSTAFSON (11) has just been read. In tomatoes dur-

ing ripening he finds a rise in respiratory quotient to a value above unity, which is thought to be caused by a shift in carbohydrate-acid metabolism. He gives some interesting speculation on the meaning of the quotients found, but concludes that further analytical data are required for adequate interpretation of results.

### Summary

1. An apparatus is described which permits aliquot parts of gas streams to be collected from the respiration chamber at desired intervals.

2. A method is described in detail for making a seal to inclose an attached leaf or fruit in the respiratory chamber, without apparent injury to the plant.

3. Under the described experimental conditions, no diffusion of either carbon dioxide or oxygen through petioles could be detected.

4. Data presented show that a considerable quantity of fat is synthesized within the castor bean fruit.

5. The possibility of translocation of some fat is discussed, and it is concluded that more detailed analytical data are required for determining whether some type of lipid is slowly moved into castor bean fruits during periods of low respiration quotients.

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# DEVELOPMENTAL ANATOMY OF THE SEEDLING OF THE RICE PLANT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 490

CHI-TUNG YUNG<sup>1</sup>

(WITH THIRTEEN FIGURES)

## Introduction

Although the rice plant (*Oryza sativa* Linnaeus) has been investigated extensively by geneticists, physiologists, and taxonomists, comparatively little attention has been given to its morphology. BRUNS' work (6) on the grass embryo includes a brief description on the rice embryo and its development. HAAN (8) made an anatomical study on the various parts of the mature plant and the grain. The flower and caryopsis were investigated by AKEMINE (1), WEATHERWAX (15), and SANTOS (13). NOGUCHI (10) studied the cytology of the megagametophyte, fertilization, and the young embryo. The present study endeavored to cover the seedling stages.

Studies on the morphology of the grass embryo and seedling have been intensive during the past century. Various writers have had divergent interpretation of the homologies of the different structures in the embryo. Excellent reviews of the literature dealing with this subject have been given by BRUNS (6), WORSDELL (16), AVERY (3), and others. The more recent publications of ARBER (2), MCCALL (9), REZNIK (11), and BOYD and AVERY (5) indicate that the controversy is still far from being settled. The chief argument is centered on the morphological interpretation of the epiblast and the relation between the scutellum and the coleoptile.

## Material and methods

Two varieties of rice were used in this investigation, the Fortuna and the Caloro. They were obtained from the United States Department of Agriculture, Bureau of Plant Industry. Grains were

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soaked overnight for the study of the embryo. For seedlings, grains were grown in damp soil in the greenhouse and in water culture. No structural difference has been found in the two lots of seedlings. The materials were fixed in CROOKS' modification (7) of Navashin's fluid, and imbedded in paraffin. Serial sections were cut at 7-12  $\mu$ .

### Investigation

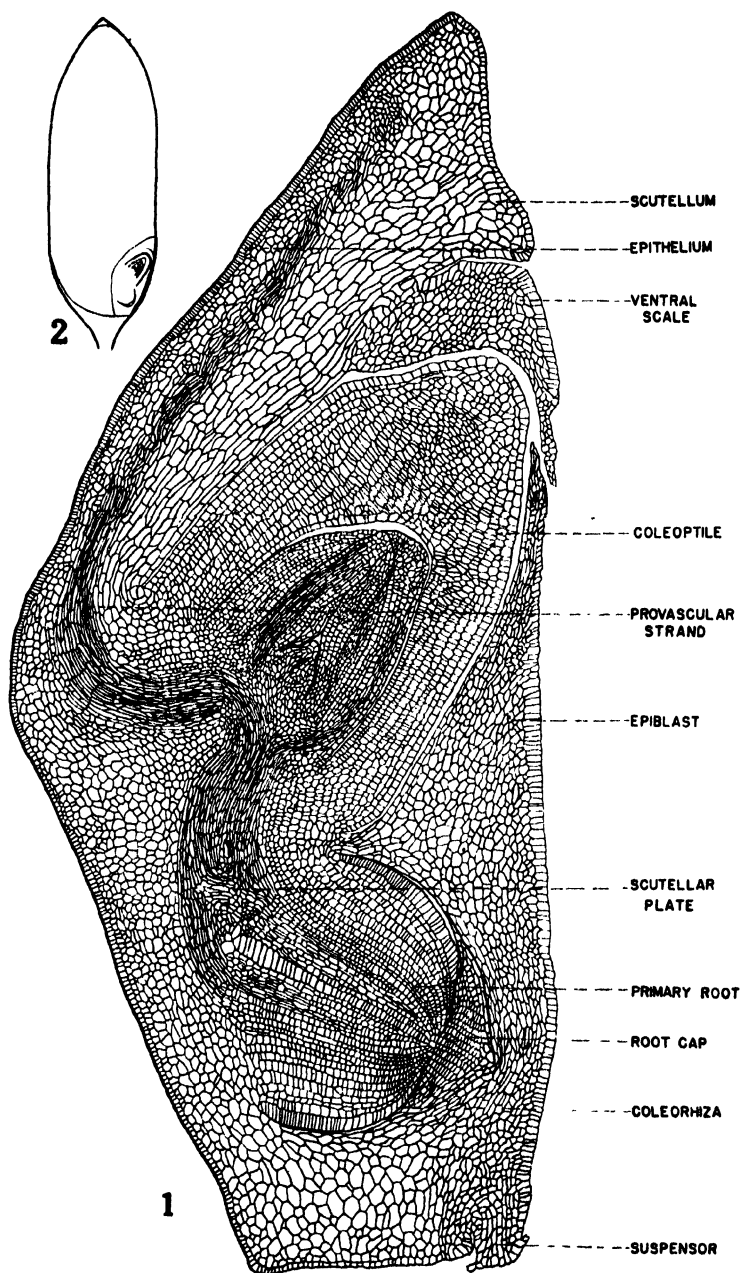
**EMBRYO.**—The embryo lies at the base of the grain on one side of the endosperm, in a slanting position (figs. 1, 2). In longitudinal section it has the shape of a semi-pentagon, two of its sides being in contact with the endosperm. In cross section it is spindle-shaped. The central axis of the stem and the root is conspicuously curved. The elongated cotyledon lies above the epicotyl, with an outgrowth from its under side, the ventral scale. The cone-shaped epicotyl is enveloped by the coleoptile. The hypocotyl, consisting of the primary root and the coleorhiza, occupies the basal part of the embryo. A large epiblast is present approximately at the level of divergence of the cotyledon. Its lower end is continuous with the coleorhiza. The upper free end extends near to the top of the coleoptile and is overlapped by the overhanging ventral scale. The latter and the epiblast inclose the coleoptile.

The cotyledon or scutellum consists chiefly of parenchymatous cells. Its surface next to the endosperm consists of a conspicuous layer of rectangular epithelial cells (fig. 1). Epithelial glands, or the infoldings of the epithelial layer, are not found. Cells of the ventral scale, epiblast, and coleorhiza, although smaller in size, are similar to those making up the bulk of the cotyledon.

The apex of the stem axis has a small growing point which together with the embryonic leaves is completely inclosed by the cone-shaped coleoptile. Near the tip of the latter there is a slitlike opening. The cells of the coleoptile and the embryonic leaves are regularly arranged in tiers. The cells at and slightly below the divergence of the coleoptilar bundle are highly meristematic; their activity during germination results in the elongation of the first internode.

The primary root is the only seminal root present in the resting embryo. It lies approximately at a right angle to the axis of the





FIGS. 1, 2.—Fig. 1, median longitudinal-side section of embryo. Fig. 2, same, of caryopsis, showing location of embryo.

epicotyl. The apical meristems are protected by the root cap, which in turn is enveloped by the coleorhiza (fig. 1). HAAN (8) described the histogens of the rice root as being the type commonly found among Gramineae. Three histogens are present. The distal one is the calyptogen; the middle layer functions as a dermatogen and also gives rise to the periblem; and the upper layer gives rise to the plerome. Such a differentiation has been attained already in the embryo. A cross section made at a higher level of the root shows a differentiated epidermis and exodermis. Cells of the inner cortex are regularly arranged radially. Endodermis and pericycle are also well defined by their arrangement as single cell layers. None of these cells has lost its cytoplasmic contents, however, nor has the wall thickened.

The provascular tissue of the embryo is a continuous strand occupying the central part of the curved axis (fig. 1). The upper and lower free ends supply the scutellum and the primary root respectively. The axis of the epicotyl contains the stelar bundle, about half of which is continuous with the cotyledonary bundle. The latter diverges abruptly from the main strand near the coleoptilar node (second node), turns downward in the cortex to form the cortical bundle, and supplies the scutellum. In a cross section at the middle of the epicotyledonary axis, therefore, two bundles are found, the stelar and the cortical.

In the embryo the first internode is very short, having the same cellular structure as the upper part of the first node. As the cortical bundle bears away from the stelar bundle there arises from it a pair of coleoptilar bundles, one on each side and at right angles to the W-shaped main provascular system. Each bundle extends first laterally for a short distance and then turns upward nearly to the tip of the coleoptile. The provascular strand to the first foliage leaf diverges from the stelar bundle also at the same level. It constitutes the midrib of the first foliage leaf and lies in the same plane as the main system; that is, at a right angle to the coleoptilar bundles.

The provascular system for the midrib of the second foliage leaf is also present. At a level slightly higher than that of the divergence of the coleoptilar bundles the basal end of the strand forks into two branches; one is united with the left and the other with the right

side of the stelar bundle at the point where the cortical bundle diverges.

The provascular system in the embryonic primary root consists of a central row of parenchyma surrounded by six or seven alternating strands of immature xylem and phloem cells. These cells are distinguishable by their size and staining reaction. The central parenchyma becomes a row of vessel segments during germination.

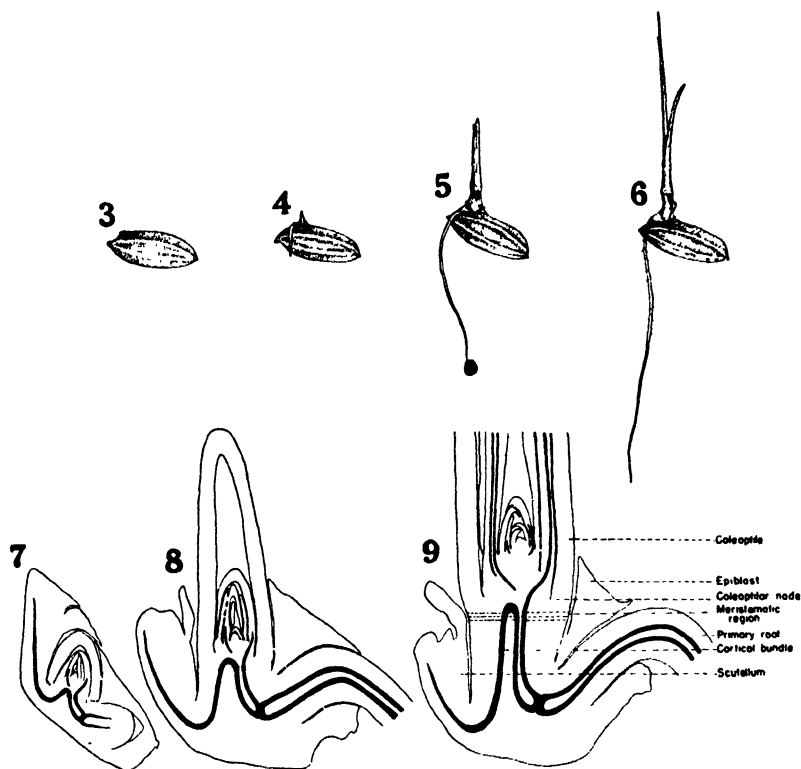
There is a vascular plate connecting the strand of the primary root with the stelar bundle. It is at this point that an abrupt root-stem transition takes place. The central row of large parenchyma branches here into two rows. Gaps filled with smaller parenchyma are found between the anastomoses of the various provascular strands forming the pith. The plate is clearly shown in the longitudinal section (fig. 2).

The cotyledonary bundle branches into small strands at the upper distal end of the main trunk. These branchlets extend backward for a short distance toward the base of the cotyledon. The lower portion of the main cotyledonary bundle does not branch at all. There is no provascular tissue in the ventral scale nor in the epiblast.

Occasionally some of the cells of the provascular strands in the embryo have secondary spiral thickenings.

**GERMINATION.**—Germination of the rice grain (figs. 3-9) is very much like that of the oat. Under favorable conditions the coleorhiza pushes through the pericarp, leaving a cavity in front of the root cap. The primary root soon elongates and fills the cavity. Subsequently the coleorhiza is penetrated. Upon further growth the root extends upward toward the epicotyl for a short distance before it responds positively to the stimulus of gravity. About this time the coleoptile also emerges and rapidly elongates. The cells of the meristematic region just below the divergence of the cortical bundle divide actively. As a result the part of the epicotyl below this divergence with its vascular bundles becomes greatly lengthened (figs. 7-9). The extent of this elongation varies according to the depth the grain is planted. When the base of the coleoptile has been brought to the upper level of the soil, the opening at its side opposite the scutellum splits more widely and subsequent foliage leaves soon appear.

In about two days primordia for two adventitious roots begin to differentiate in the meristematic zone on the side opposite the scutellum. They soon penetrate through the cortical region. A third adventitious root may appear later at the coleoptilar divergence opposite the two older ones. Lateral roots soon develop from the primary and adventitious roots.



FIGS. 3-9.—Figs. 3-6, stages of development in germination. Fig. 7, median longitudinal-side section of dormant embryo. Region in which first axial elongation will begin shown in dashes. Fig. 8, germinating embryo, 48 hours old, showing beginning of germination. Fig. 9, same, 4 days old.

**SEEDLING AXIS.**—HAAN (8) studied the vegetative structures as well as the spikelet and mature grain of rice. His description of the root, stem, and leaf was based chiefly on mature plants. He has not mentioned the vascular system, especially that of the seedling stages. A brief description of the various organs in addition to a

detailed account of the vascular skeleton of the young rice plant therefore seems desirable.

All roots, primary, adventitious, and secondary, generally are hexarch radial protostele. Roots with seven xylem strands are not infrequently found. The epidermis consists of a single layer of cells. Immediately beneath this region there is a layer of collenchyma forming the exodermis. The cortex is the most prominent part of the root; the outer one or two layers of cells have their walls greatly thickened. The large inner parenchymatous cells are filled with starch grains. The endodermis is well defined by the thickened inner tangential wall. The pericycle consists of one layer of cells.

The part of the axis between the scutellar and coleoptilar divergence in the grasses is termed the "first internode" by BOYD and AVERY (3, 4, 5). This region in rice is well marked and elongates appreciably during germination. The homology of this organ will be discussed later. Cells of the first internode are not so elaborately differentiated as are those of the root. The epidermis is present as a single layer of cells. Exodermis is wanting. The cortex consists of a wide strip of thin walled parenchyma. Imbedded in the cortex on the side next to the scutellum is the cortical bundle which runs downward and backward to the scutellum. In older seedlings air spaces are present in the cortex. The endodermis and pericycle are single layers of thin walled cells. The vascular tissue in the stele resembles that of *Avena* (3). It consists of four bundles. Two large endarch bundles are separated by a small patch of parenchyma which may be termed the pith (fig. 11C-D). One of the endarch bundles is a direct continuation of the cortical bundle. It is termed the "common bundle" by BOYD and AVERY (5) because of its relation with the coleoptilar bundles and the lateral bundles of the first foliage leaf. The other endarch bundle runs directly into the midrib of the first foliage leaf. The arrangement of the xylem elements in the lateral bundles is neither endarch nor exarch. In young seedlings their identity is lost at the meristematic zone just below the coleoptilar node. It is also at this level that the first two adventitious roots arise.

At the scutellar plate (fig. 11D-F) each of the two endarch bundles of the first internode is formed by fusion of two adjacent bundles of the hexarch stele of the primary root. Each of the remaining

unfused bundles of the root has two side branches, one on each side, which are joined to the two endarch bundles; these extend upward as the two lateral bundles of the first internode. In this manner the six bundles of the root are reduced to four in the first internode. The vascular plexus is made more complex by the fact that it occurs at the notch where the vascular skeleton of the root and stem form

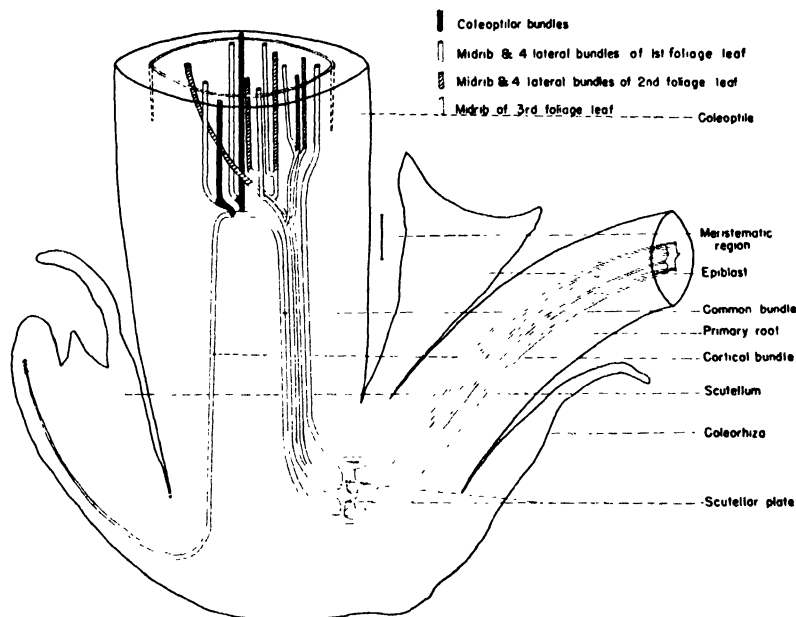


FIG. 10.—Diagrammatic reconstruction of lower portion of seedling showing principal structures present, and vascular interrelations of scutellum with axis, coleoptile, and first foliage leaves.

an acute angle. When seven xylem groups are present in the root, one of the endarch bundles is anastomosed with three instead of with two bundles, as in the case of *Avena*.

At the level of the coleoptilar divergence the following main vascular bundles are found (figs. 10, 11B): (1) a pair of coleoptilar bundles; (2) a midrib and two pairs of lateral bundles for the first foliage leaf; (3) a midrib and two pairs of lateral bundles for the second foliage leaf; (4) a midrib for the third foliage leaf. The relation between these bundles and the four of the first internode is as follows.

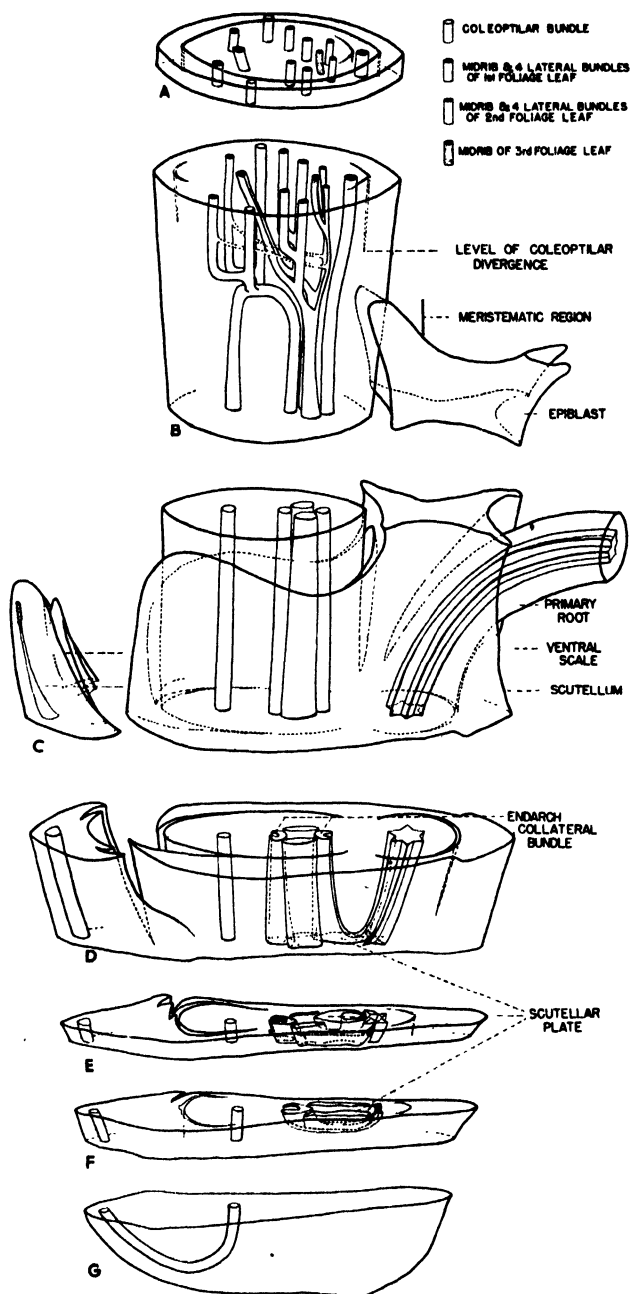


FIG. 11.—Three-dimensional diagrammatic presentation of fig. 10, showing detailed course of vascular bundles and various organs of seedling.

(1) Each of the two coleoptilar bundles is fused with an outer lateral bundle of the first foliage leaf which lies on its scutellar side and then joins the cortical bundle at the point where the latter bears away from the common bundle of the first internode.

(2) The midrib of the first foliage leaf is a direct continuation, without branching, of the endarch bundle opposite the common bundle.

(3) Each inner lateral bundle of the first foliage leaf is fused with the adjacent lateral bundle of the second foliage leaf and then with half of the midrib of the first foliage leaf. The resulting bundles then turn downward and meet the lateral bundles of the first internode at the meristematic zone.

(4) Each of the remaining two lateral bundles of the second foliage leaf is fused with half of the midrib of the third foliage leaf before they join the lateral bundles of the first internode at the meristematic zone.

(5) There is a vascular anastomosis at the second node, linking the lateral bundles of the first and second foliage with the midrib of the second foliage leaf (fig. 11B).

**COLEOPTILE.**—The point of divergence of the coleoptile and the origin of its two vascular bundles have been described. It consists chiefly of closely arranged parenchyma. In cross section cells of the outer epidermal layer are smaller in size and slightly rectangular in shape. Stomata are present, especially near the apex. Under very moist conditions exudation takes place in these pores. Few chloroplasts are present. In all the specimens studied, no coleoptile has been found to possess more than two vascular bundles. The bundles are placed approximately opposite each other, in a plane at right angles to the midribs of the later foliage leaves. Each bundle in cross section consists of a row or two of sclerenchymatous cells on the outer side. Phloem cells occupy a little more than half of the total bundle area. Xylem cells are relatively few and are arranged in a row adjacent to the inner side of the phloem. A bud primordium is present in the axil of the coleoptile (the side toward the scutellum). Rarely this primordium develops.

**FOLIAGE LEAVES.**—A mature foliage leaf consists of the sheath at the base which surrounds the culm for some distance, the blade

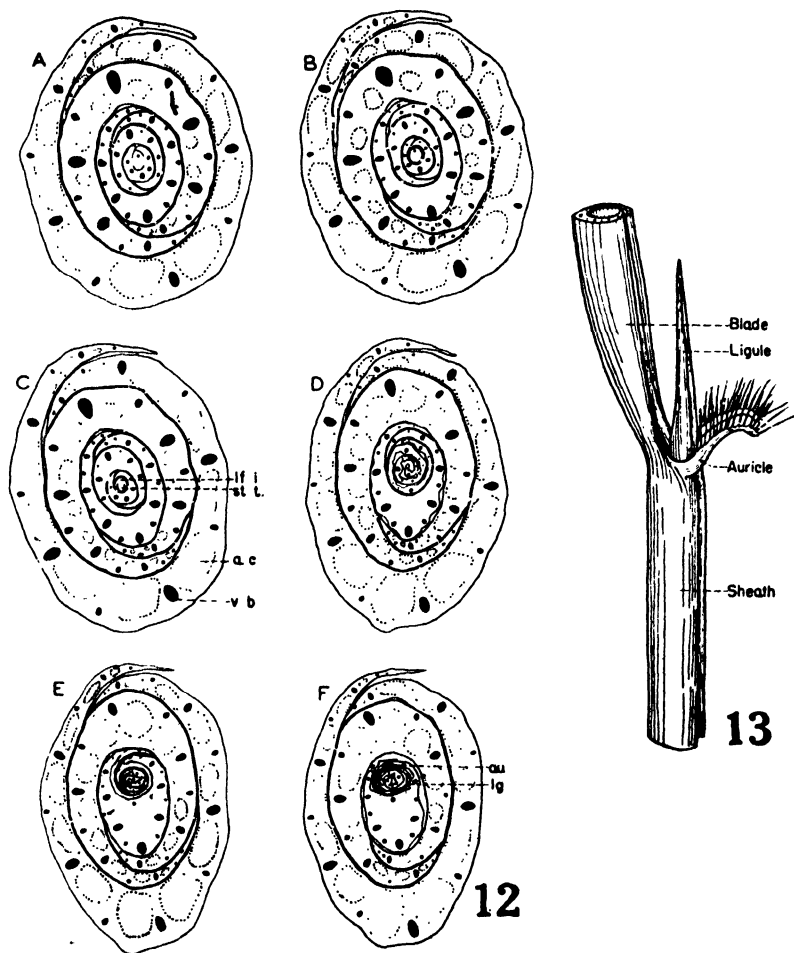


which is set at an angle with the culm, the ligule, and the auricles (fig. 13). Descriptions of these structures are given by other workers (2, 8).

RÖSELER (12) reported that in wheat the leaves originate from the dermatogen alone. This may also be true for rice, but the evidence cannot be considered conclusive until further investigation is made. The leaf primordium begins to develop at a point near the growing tip of the stem on the side opposite the midrib of the preceding leaf (fig. 12C). Soon cells at either side of it are differentiated from the stem in similar manner until the two edges of the primordium overlap, thus inclosing the growing stem tip. After two or three tangential divisions of the initial cells, a dermatogen (tunica) is differentiated. This is followed by the differentiation of the provascular tissue, first the midrib and then the lateral bundles. The primordium increases rapidly in length, and soon the sheath is visibly distinguishable from the blade. In the early stages of leaf formation the blade grows much faster than the sheath. Frequently the blade has attained almost full length while the sheath is only an inch or two in height.

There is a radial thickening in the sheath around the central leaf axis beginning at the point just above the growing tip of the stem (fig. 11D-F). This thickening is the result of the repeated tangential divisions of a row of cells under the inner epidermis in the direction of the midrib. The leaf is thickest at the base of the blade (fig. 12F). In the blade the thickening decreases gradually, and finally appears as a ridge containing the midrib and projecting from the abaxial surface of the leaf.

The ligule develops on the adaxial face of the leaf while the latter is still young and the cells are still meristematic (fig. 12E-F). The upper margins of the sheath are continuous with the overlapping margins of the ligule. The median portion of the ligule is an outgrowth from the adaxial epidermis of the leaf. The development of the ligule begins by a tangential division of some adaxial epidermal cells at the apex of the sheath. The new daughter cells soon split from the sheath. The splitting gradually extends laterally toward the abaxial surface of the sheath, thus incorporating the newly formed outgrowth with the inner border of the sheath to form the



FIGS. 12, 13.—Fig. 12, cross sections of epicotyledonary axis: *A*, near stem tip, showing four encircling sheaths (fifth one not yet separated from stem); *B*, 12  $\mu$  above *A*; *C*, 24  $\mu$  above *B*, showing leaf initial (*lf.i*) being differentiated from stem tip (*st.t*); *D*, 1.6 mm. above *C*, showing blade surrounded by three sheaths; *E*, 1.1 mm. above *D*, showing lower end of ligule; *F*, 0.12 mm. above *E*, showing ligule (*lg*) completely detached from leaf proper and upper end of auricles (*au*). Fig. 13, middle portion of leaf.

ligule (fig. 12E). In a fullgrown leaf the ligule is in a plane continuous with the axis of the sheath, while the blade is at an angle to this axis. The auricles are developed at a slightly higher level than the ligule. They first appear as two knoblike outgrowths from the base of the blade, one on each side of the lamina. Upon further growth they increase in length and long hairs develop on their edges. Where the blade bears away from the sheath, the auricles bend slightly in the opposite direction and encircle the culm.

There are small cross veins connecting the parallel bundles of the leaf sheath and blade. These veinlets begin to differentiate while all the cells are still meristematic. At more or less regular intervals single rows of cells between two adjacent bundles begin to divide. Each cell divides tangentially two or three times. Usually the cross walls are not formed until the entire series of divisions is completed. The result is that each original mother cell gives rise to a bundle of elongated cells, and these long daughter cells are joined end to end with those of the adjacent cell. In cross sections of young leaves, such chains of provascular tissues are plainly seen linking up the parallel veins.

The presence of air cavities in the leaf is characteristic of the rice plant. These cavities are large and more or less cylindrical with thin diaphragms as cross partitions. In the diaphragm small cross veins are invariably found, often forming an anastomosis. The formation of these cavities begins when the cross veins start to differentiate. Groups of cells in the leaf at regular intervals very close to each other cease to become meristematic. The cell contents disintegrate and the cell walls lose their elasticity. As the leaf continues to elongate by division and enlargement of the other cells, these degenerate ones are pulled apart, forming cavities between the living cells; the result is like a honey-comb structure in the leaf. These cavities continue to enlarge as long as elongation of the leaf takes place.

Generally, as soon as the formation of an air cavity is started, all cells except those near the base of the leaf sheath constituting the intercalary meristem begin to lose their capacity of cell division. They enlarge only in size. The provascular tissue matures into xylem and phloem elements. Sclerenchyma is differentiated. The intercalary meristem continues to give rise to new cells, adding length to

the sheath until the latter is almost as long as the blade. The new cells thus derived repeat the processes of tissue differentiation and maturation as already described.

### Discussion

The rice embryo closely resembles that of the oat. They both have similar arrangement of the provascular system and general structural makeup. The position of the meristematic zones is identical in both embryos. Some points of difference are: (1) the stem-root axis of the rice embryo forms a sharp angle, whereas the axis of oat is a straight line; (2) the rice embryo has a larger epiblast and is overlapped by the ventral scale, thus completely inclosing the coleoptile; (3) seminal roots are absent where the epiblast diverges, a fact which may be related to the curved nature of the stem-root axis.

The vascular system of the rice seedling also agrees with that of the oat seedling. Both possess the inverted cotyledonary trace which runs backward and downward in the cortex all the way through the first internode. In the rice seedling each of the coleoptilar bundles is united with only one lateral bundle of the first foliage leaf, but each coleoptilar bundle of the oat is united with two lateral bundles of the leaf above. This minor difference does not mar the close resemblance.

ARBER (14, 2) and earlier investigators have advanced a hypothesis in an attempt to homologize the scutellum of grasses with the blade of a foliage leaf, the coleoptile its ligule, and the intervening axis, "mesocotyl," as an elongated cotyledonary node. This theory assumes that the scutellum has a closer vascular relationship with the coleoptile than with the later foliage leaves. BOYD and AVERY (5) have shown that such an assumption is not in accord with the situation found in the oat. The rice seedling is similar to that of the oat. The common bundle in the first internode (ARBER's mesocotyl) supplies two lateral bundles of the first foliage leaf as well as the coleoptilar and scutellar bundles. BOYD and AVERY cite the case of *Billbergia zebrina* and four other genera of Zingiberaceae to substantiate their contention that the scutellum and the coleoptile are separate organs, one being the cotyledon and the other the sec-

ond leaf. The cotyledon of *B. zebrina* has a well developed ligule, the divergence of which is far below the vascular fusion of the cotyledonary trace and the bundles of the first foliage leaf (equivalent to the part of the axis just above the meristematic zone in oat and rice). In no way can the first internode be interpreted as an elongated node. In view of this strong evidence, the writer feels justified in accepting BOYD and AVERY's interpretation and applying the same to the findings in rice; that is, the scutellum is the first (seed) leaf, the coleoptile the second leaf, the first foliage leaf the third leaf, and so forth. Other arguments for and against this interpretation have been fully discussed by others (2, 3, 4, 5, 6, 9, 14, 16).

MCCALL (9) recently revived the old controversy that the epiblast is a second cotyledon. In his study of the wheat seedling he found a vascular anastomosis which he considered as an extra node in the axis between the scutellar and the coleoptilar divergence (first internode of AVERY). In the rice seedling, as in the case of oat, this vascular anastomosis is situated far above the scutellar divergence and has no relation to the scutellum. While the absence of vascular tissue in the epiblast is only a negative evidence and does not exclude the possibility that the epiblast is a second cotyledon, stronger positive evidence is still needed to prove that the epiblast in any case is a vestigial second cotyledon.

### Summary

1. The embryo and the development of the seedling of the rice plant are described.
2. The general structural makeup and the provascular system of the rice embryo closely resemble that of the oat.
3. The primary root is the only seminal root present in the embryo. It is commonly a hexarch radial protostele, and lies approximately at a right angle to the axis of the epicotyl. It has three well defined histogens as commonly found among the Gramineae.
4. The root-stem transition of the vascular system takes place at the scutellar plate.
5. The scutellum is the cotyledon with a single cotyledonary (scutellar) bundle which branches only at its upper distal end.
6. The first internode contains four bundles. The relation of these

bundles with those found at the second and higher nodes is described.

7. There is a meristematic region just below the second node. The activity of the cells in this region is responsible for the elongation of the first internode during germination.

8. The coleoptile is the second leaf. Its two vascular bundles have the same relation to the cotyledonary bundle as a pair of lateral bundles of the first foliage leaf.

9. There is no vascular tissue in the epiblast, ventral scale, and coleorhiza.

10. The development of the foliage leaves is described.

The writer gratefully acknowledges the valuable assistance of the members of the staff of the Department of Botany of the University of Chicago during the progress of this work.

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## STRUCTURAL PROBLEMS AT THE MERISTEM<sup>1</sup>

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Problems of development dominate biology today. For the morphologist, the physiologist, and the geneticist it is the growing and differentiating organism, unfolding its innate capacities as it develops from fertilized egg to maturity, which occupies the center of attention. The adult plant or animal, no matter how carefully studied, cannot be understood by itself but must be interpreted by the aid of a knowledge of those stages and processes which are responsible for its final state. Such an analysis is evidently a very difficult task, and involves far more than a study of descriptive embryology alone. It is becoming the concern of almost every student of biology, and has called to its aid the biochemist and the biophysicist.

For this attack upon the problems of development, botanists are in a peculiarly favorable position. Plants are rather rigid structures and their cell walls are in general thicker than those of animal tissues. As a result, the typical mature plant cell can neither divide nor grow. Unlike the animal, therefore, the plant cannot enlarge throughout all its parts but must depend for this function on localized groups of undifferentiated and perpetually embryonic cells, the "growing points" or meristems. Meristems may briefly be defined as any regions where cell multiplication occurs. They are widely distributed in the plant body and many of them continue to function indefinitely. By their activity there arises a constant series of new organs or constant increments to old ones. Development in a plant is therefore not a single history but a continuing series of indefinitely recurring ontogenies. Its processes may be studied repeatedly in the same individual and under as wide a range of environmental conditions as the investigator can control. These advantages, together with the relatively simple character of plant

<sup>1</sup> Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.



structure and organization, make meristematic tissues particularly favorable material for the study of developmental problems.

Despite their importance, our knowledge of the structure and activities of meristems is surprisingly meager. Early workers, following HANSTEIN'S (10) promulgation of the histogen theory in 1868, endeavored to distinguish definite germ layers in the meristem. The failure of this attempt, together with a shift of botanical interest to other fields, resulted in comparative neglect of studies of the meristem for many years. More recently, however, there has been a marked revival of interest, both theoretical and practical, in such problems. No longer are we concerned primarily with the morphological equivalence of particular layers or the variation in number and shape of initial cells in different plant groups. Instead we wish to find just what determines the point of origin and the progressive development of an organ primordium; what decides whether a vegetative shoot or a flower shall be formed; what factors control tissue differentiation; and what produces differences in growth rates in the various dimensions and thus molds the form of a developing organ. The meristem is the strategic point where the fate of the plant is primarily decided, and upon this point the attention of botanists is coming more and more to be centered.

Doubtless the answers to the problem of the meristem will ultimately be given in physiological terms, but some of the most difficult ones can best be stated at present in the language of form, size, and structure. Certainly before their solution can be intelligently attempted it is essential that a detailed description, quantitatively expressed, be given for the various changes in cells and tissues which take place as the organs of the plant develop from their meristematic beginnings. The purpose of the present paper is to formulate a few of the more important of these problems in terms of structure. Their investigation is the perquisite of no one branch of biological science but requires cooperation from every field.

It should first be recognized that there are important differences in the localization and duration of meristematic activity. Some meristems are self perpetuating, whereas others are definitely limited in their duration and produce structures rather rigidly determinate as to size and shape. The former, represented in the higher plants by

the terminal meristems of root and stem and by the cambium, has received far more attention among botanists than the latter, and its activity has often been pointed out as the most distinctive feature of plant growth. One should remember, however, that only the axial parts of the plant body—the roots and stems—enlarge by means of growing points with indeterminate activity. Two important organs, the leaves and the reproductive structures, with their various parts, are definitely limited in growth and arise from diffused meristematic activity which is not self-perpetuating but ultimately ceases entirely as the organ attains maturity. In the leaf this process is relatively brief, but a much longer time may elapse between the young floral primordium and completion of its growth into a mature fruit. In both the process is essentially the same. A tiny primordium, meristematic throughout, enlarges by cell multiplication and is differentiated into various parts and tissues. In some of these cell division ceases early and in others persists longer, but in all it ultimately comes to an end. After a varying amount of cell enlargement the organ then stops growing. The existence of indeterminate meristems is associated with the very loose, semi-colonial character of the plant body, which usually consists of an indefinite multiplication of similar parts or structures. Determinate meristems, on the other hand, develop in a manner much more like that of an organ of an animal, and similarly lead to the production of specific forms or patterns. Indeed the question of form determination is here presented in one of its simplest expressions, so that this type of meristem is particularly favorable for morphogenetic investigations.

Some of the fundamental problems raised by a study of the activity of both these types of meristems can be formulated most simply in terms of individual cells. Of especial significance are those concerned: (1) with cell division, (2) with cell enlargement, (3) with cell polarity, and (4) with cell shape. Each will be considered briefly in turn.

1. Mitotic cell division is the basic process underlying all meristematic activity, but it is by no means uniformly distributed. In secondary meristems it is sharply localized in a thin cambial layer. In primary meristems of root and stem it is most frequent at the tip and becomes progressively less so with increasing distance back

along the axis, until it finally ceases. In determinate meristems it is variously distributed but in some regions is more active and persists longer. Thus in certain leaves a marginal zone of dividing cells persists after mitosis has ceased in the bulk of the leaf. In the ovary of the cucurbits the entire primordium is at first meristematic, but division soon ceases in the central tissue, then progressively in the inner wall layer and outer wall layer, and finally, not long before maturity, in the epidermis. In such meristems the location and rate of cell division are evidently very important in determining growth and development.

There is also considerable evidence of a regular distribution of mitoses in time. Several students of root meristems have reported rhythmically alternating periods of high and low mitotic rate (5), the latter associated with more rapid cell enlargement. WAGNER (24) finds evidence also of a spatial rhythm at root growing points, a careful census showing two or sometimes three zones of higher mitotic rate at definite distances back from the tip.

Evidently the basic problem underlying all this is concerned with the factors which induce or prevent the process of division. The literature on this problem, both for animals and plants, is extensive and conflicting, and there is no agreement as to the paramount importance of any single factor. Cell size evidently plays a vital part, for small cells divide more rapidly than large ones, and beyond a certain size (specific for tissue or organism) division does not take place. As a cell increases in volume, its surface becomes smaller in proportion to its bulk. Mitosis has been regarded by VERWORN (23) and others as a mechanism for restoring an optimum relationship between these two quantities. ABELE (1) looks upon the surface-volume relationship of the nucleus rather than of the entire cell as determinative. HERTWIG (11) and others saw in the changing relation of nuclear to cytoplasmic volume the primary stimulus to mitosis. Just how all these various ratios actually effect the induction of mitosis is not clear.

Various chemical and physical factors have also been stressed as of primary importance in causing cell division. Mitogenetic radiations, long a subject of controversy, are still believed by some workers to be an important factor. Protoplasmic viscosity, as shown by

the work of KOSTOFF (14), FRY and PARKS (6), and others, is much lower during mitosis than in the resting cell; but whether this change is cause or result is not clear. The work of HAMMETT (9) and his school has emphasized the importance of the sulphhydryl ion in stimulating division, presumably because of its role in nuclear respiration. Specific chemical substances, such as the wound hormones of HABERLANDT (8) and the auxins of more recent workers, seem to be definitely related to the mitotic process, at least in certain cases, although here again it is not certain whether they are inducing agents or products. Students of cellular physiology will evidently make a contribution of profound importance to all problems of development when they discover what are the ultimate factors which cause a cell to pass from the resting to the dividing state.

2. An understanding of the process of cell division alone will not solve all the problems of the meristem, however, for cells increase in size as well as in number. Students of plant growth have long recognized two rather sharply distinct phases, an early one of cell multiplication and a later one of cell enlargement only. The latter process, during which the bulk of the cell may be enormously increased, is a conspicuous and highly important aspect of growth. The extent to which the various cells enlarge determines the size of the organ and the degree of differentiation within it. This phase of growth is not in the strict sense a part of meristematic activity, however, since it occurs after cell division ceases.

There is a less conspicuous but a constant and significant increase in cell size during the period of cell division. It is remarkable how little attention this fact has received from students of the meristem. The common idea that there is a rather specific, presumably optimum, cell size for mitosis is incorrect, for in all types of meristems except cambia (and perhaps there) it seems to be the general rule that minimal cell size occurs in the youngest tissue—the terminus of the meristem or the earliest primordium—and that there is a steady increase until a rather definite size is reached, after which mitosis ceases and a much more rapid and conspicuous cell enlargement begins. This can best be seen in a simple terminal meristem like that of a root, where in passing from the apex back through the mitotic zone, an increase in cell volume amounting to several

hundred per cent often occurs. In cucurbits the tiny primordium from which an ovary develops is made up of very small cells; but as growth proceeds these cells, all meristematic, increase steadily in volume, until just before mitosis ceases they sometimes reach fifty or more times their initial volume. In a given tissue there is usually a constant relationship between the rate of this cell expansion and the rate of cell division. Where expansion is relatively rapid, the maximal cell size and the cessation of division occur early and total cell number is thus relatively small. Where expansion is slower in proportion to division, there are more divisions before mitosis ceases, and the total cell number, and consequently the ultimate size of the organ, is much greater. Similarly, where the maximal cell size for mitosis is high, there will be opportunity for more cell divisions before it is attained, and thus greater ultimate organ size, than where the maximal size is lower. If cell size is the limiting factor in cell division, the particular maximum for a given tissue or organ and the rate of expansion to this maximum are clearly very important factors in determining the ultimate size of the structure concerned.

It should be remembered that a somewhat similar phenomenon occurs even in the cambium, for SANIO (20)—essentially confirmed in this respect by BAILEY and TUPPER (2)—found that there is a continuous increase in the length of wood cells, and thus presumably of their cambial initials, for many years.

The cause of cell expansion is unknown but is obviously a part of the basic problem of growth itself. Evidently in most cases each progressive pair of daughter cells reaches a somewhat greater size than their mother cell before division again intervenes. When division ceases, this expansion, unchecked, is much more rapid and conspicuous. In its later stages enlargement seems entirely due to absorption of water. How much of it in the earlier cases results from this cause and how much from actual increase in living material is unknown, and remains to be determined by the cell physiologist.

3. But the problems of cell division and cell expansion are not the only ones which must be solved before an understanding of meristematic activity can be attained. The plane in which division occurs is also important. Mitotic figures are not random in their

orientation, but are usually more frequent in one direction or dimension than in another; indeed this is the primary cause behind the development of those specific shapes which are so characteristic of organisms. Whatever controls the direction of cell division ultimately controls organic form.

In meristems of indeterminate growth the problem is relatively simple, since division is mainly in one direction. At the very tip of the growing point of root or shoot, division is irregular, but soon most of the new walls are laid down at right angles to the axis, producing the cell rows or layers of essentially rectangular cells so characteristic of such tissue, and which convinced HANSTEIN and his followers that definite germ layers existed here. Similarly, in the cambium the great majority of the divisions are in the tangential or periclinal direction, resulting again in characteristic cell rows. Why in all these cases the cells should be more nearly four-sided than six-sided in section raises another problem, and suggests that the longitudinal walls (in primary meristems) or the radial ones (in cambia) are firmer than the walls at right angles to them.

It is in the determinate, organ-forming meristems that the problem of the plane of cell division assumes much more significance, since from these meristems structures of specific shape develop. If one makes a developmental analysis of such a structure, as of an ovary primordium, it becomes evident that cell division proceeds more rapidly in certain directions than in others. Thus in most cucurbit ovaries the wall grows much faster in length (axial dimension) than in width (radial), and this difference is found to be due to a much more rapid division rate in the former dimension than in the latter. The cells are not in rows, and the divisions are at many different angles with respect to the axis rather than merely in two; but the general average preponderance in one direction rather than in the other is so accurately maintained that the ratio between the two dimensional growth rates is constant. At the same time the internal ovarian tissue is growing more rapidly in width than in length, owing again to the more rapid division of its cells in one plane than in the other. In fruits of cucurbits there are constant differences in relative growth rates between length and width of ovary, width of placental tissue and of wall, length and width of

"neck," and width of ovary and "neck," all these differences being determined by corresponding differences in the frequency of cell divisions in various directions.

As to what determines the plane of cell division, there are again many hypotheses but no certain knowledge. The "rules" of HOFMEISTER (12) and of SACHS (19) that the wall between two daughter cells always shows a constant orientation to the axis or to the wall of the mother cell, hold good in many cases but are far from true in others. The suggestion that the position of the new wall is essentially like that which would be assumed by a weightless liquid film, proposed by ERRERA (4) and by BERTHOLD (3) and elaborated by THOMPSON (22), is physically simple and satisfying, and is undoubtedly applicable in certain cases; but there are many others which it cannot explain and it clearly leaves out of consideration a number of important biological variables.

The problem of plane of cell division evidently involves primarily the polarity of the cell, or more probably, as GIESENHAGEN (7) suggests, the polarity of the nucleus. Whatever it is that orients the mitotic figure must be the chief factor in determining where the new cell wall is to be laid down, and thus the direction of growth of the two daughter cells. Various factors have been suggested as determinative. The first division in isolated cells is usually at right angles to gravity. It has been shown by KNY (13) and others to be parallel to the direction of applied pressure, and by STAHL (21) and others to be at right angles to incident light. More recently LUND (16) has stressed the importance of electric currents in determining polarity. WHITAKER (25, 26) and his students (15) have shown that the position of neighboring cells, the visible cell constituents as rearranged by centrifuging, and gradients in temperature and in hydrogen ion concentration, all are related to the induction of polarity; and OLSON and DU BUY (18) have found auxin similarly effective. Most of these investigations have been carried on with single cells, notably the eggs of *Fucus* and spores of various sorts. What seems especially needed is similar work with masses of meristematic cells, toward which the studies of NEEFF (17) on the experimental alteration of the polarity of cambial cells point the way. When the cell physiologist can determine precisely what are the ultimate factors

which control cell polarity, and how they operate, he will have made a significant contribution to our understanding of development. It is at this point that physiology and morphology come most closely into contact, and here seem to focus some of the ultimate problems of biological science.

4. The fourth problem is that of change of form among meristematic cells. Most of these are isodiametric, and the bulk of change in cell shape occurs during the process of differentiation after division has ceased. Even in the meristematic tissues, however, there are the beginnings of considerable cellular differentiation. Thus the elements of the provascular strands consist, even while division among them is still going on, of cells markedly elongated parallel to the axis of the organ. In relatively small ovary primordia of cucurbits the inner layer of the wall consists of cells elongated parallel to the axis, and the outer layer of cells tangentially elongated. Both types become isodiametric later but are far from being so in their early stages. In all such cases the suggestion is obvious that cell polarity is also involved. The role of plant hormones in determining cell elongation through their effect, direct or indirect, on wall growth has been studied by many recent workers and provides the only definite suggestion at present for the origin of differences in cell shape.

These four major structural problems in the meristem: cell division, cell enlargement, cell polarity, and cell shape, have all been approached here from the point of view of the cell rather than of the cell aggregate. There remain the more complex matters, such as the origin of leaf primordia through the more rapid growth of surface layers in comparison with those beneath, the change in form of the meristem during the course of a single plastochrone, or the differences in the shape of the meristematic cone which are associated with the production of primordia of vegetative and of reproductive organs respectively. With these should also be considered the important physiological studies which have concerned themselves with meristematic changes. The effects of mineral nutrients, of differences in light intensity and in photoperiod, of carbohydrate-nitrogen relation and of growth-differentiation balance, are among the more important of these researches. Nevertheless beneath all



these more complex developmental phenomena lie those changes in individual cells to which attention has here been directed. There well may be integrative factors which control mass phenomena in development, but these must operate through changes in number, size, position, and form of individual cells. To describe precisely what happens at the meristem, in terms of the structure of cells and cell aggregates, is the important task of the cytologist and the morphologist. An ultimate solution of the problems thus formulated must depend upon many biologists, but especially upon students of cellular physiology. When through such cooperation we gain a clearer understanding of the processes which go on in these determinative regions, we shall be well on the way toward a solution of the major problems of plant growth and development.

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# RELATION OF CONDENSATION REACTIONS TO MERISTEMATIC DEVELOPMENT<sup>1</sup>

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## Introduction

Evidence from many sources indicates that vegetative apical meristems are commonly dominant in correlative plant development. Examination of an active apical meristem of a shoot or root axis shows a mass of dividing cells at or near the tip of the axis. Cells within an easily recognizable apical zone continue to divide as long as temperature, moisture, food, and related factors are favorable. Cells left behind by the advancing tip either cease division entirely or divide slowly and irregularly during the period of enlargement. The cells in the region of division are characterized by a dense, relatively unhydrated protoplasm and by the rapid rate at which they are able to condense amino acids and similar nitrogenous compounds into new protoplasm.

Meristems such as the vascular cambium, root initials during early stages of their formation, growing flowers, intercalary meristems in grasses, etc., show no comparable massing of dividing cells, and these diffuse meristems are characteristically less effective than the massed meristems in utilizing and in competing for the materials of which the cell is constructed. We suggest as a working hypothesis for explaining these reactions, that masses of meristematic cells are able to build up conditions favorable to protoplasmic condensations while similar reactions can occur in diffuse meristems only under more favorable circumstances, or with the assistance of factors external to the meristem.

## Condensation and cambial development in *Populus*

A number of workers have shown that cambial growth in deciduous trees is initiated subsequent to shoot growth, and that it starts

<sup>1</sup> Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

at the tip of the shoot. It has been shown previously (6) that cambial development in apple (*Pyrus malus* L.), poplar (*Populus nigra* L.), and boxelder (*Acer negundo* L.) was promptly arrested at any time during the growing season by removing a ring of phloem above the test region. If leaf-bearing branches were present below the ring, cambial growth continued below the branches. If the ring was made below the lowest branch, all cambial development of the shoot below the ring ceased quickly, while secondary growth above the ring proceeded for a time at a normal or accelerated rate. Chemical analyses of xylem and phloem showed that cambial division was correlated with a relatively low concentration of soluble organic nitrogenous compounds; and shoot initiation, such as occurred below the rings, with a relatively high concentration of these substances. With the three species used, one showed high mono-amino nitrogen correlated with development of massed (shoot or callus) meristems, the second showed high basic nitrogen, and the third a residual fraction neither mono-amino nor basic. Apparently any of a number of organic nitrogenous compounds may be condensed into protoplasm when conditions are favorable.

More recently AVERY, BURKHOLDER, and CREIGHTON (1) have shown that initiation of cambial growth in woody twigs is preceded by formation of growth promoting substances within the buds and its polar export down the twig. Growth substances, however, could not be isolated by diffusion from the phloem of the trunk or larger branches of trees showing rapid cambial development. They failed also to obtain a correlation between the quantity of growth substances diffused from the terminal buds and the rate of cambial development. By July 16 the growth hormone production had dropped to less than 5 per cent of its May peak, yet cambial division is commonly proceeding at a maximum rate during July and August.

In an earlier paper (6) the writer questioned the role of hormones in cambial growth because of the apparently dominant effect of mature leaves. A number of recent experiments, the details of which will be published elsewhere, have shown beyond question the possibility of stimulating cambial development in *Populus* with growth substances. Microscopic measurements have shown cambial stimu-

lation by etiolated and by defoliated shoots, as well as by normal shoots in the light, and direct stimulation of cambium, both in one-year-old twigs and in eight-year-old trunks of *Populus balsamifera* L., by a 0.2 per cent paste of heteroauxin in lanolin. Chemical studies of the regions in which cambial growth has been stimulated have shown a statistically significant reduction of the organic nitrogenous compounds soluble in 80 per cent alcohol (7) in all but one experiment. In this experiment, with small disbudded plants treated with heteroauxin, rapid upward translocation in the absence of shoot growth appears to have offset the expected decrease in soluble nitrogen. Data on growth and soluble organic nitrogen from several experiments are included in table 1. Cambial growth was varied by: (a) Cutting away the phloem above or below the test segments. (b) Position above or below a side branch on a trunk segment isolated by phloem rings; cambium developed only below the side branch in such a segment. (c) Growing in darkness and in light, with and without disbudding; etiolated shoots stimulated cambial growth but at a reduced rate. (d) Applying heteroauxin pastes. All treatments which increased cambial growth decreased the percentages of soluble organic nitrogen. Changes in total organic nitrogen showed inconsistencies associated with storage and digestion of materials not directly related to growth. The trend was toward increased insoluble nitrogen in the regions showing cambial development.

The data may be explained by assuming that the diffuse meristem, the cambium, was unable to condense the soluble organic nitrogenous compounds formed by digestion of stored proteins. With the aid of leaves in the light, growing etiolated buds (supplying auxin?), or external supplies of heteroauxin, conditions were made more favorable for condensation reactions, and the cambial cells were able to synthesize protoplasm and to divide. A causal relationship between condensation and cambium development is indicated by the suddenness with which cambial division ceases and soluble organic nitrogen concentrations rise below phloem rings cut during periods of rapid cambial growth.

It has been suggested (6) that the direct condensation of nitrogenous compounds in lighted, mature leaves may explain their effect upon cambial development. A condensing effect of auxins and the possibility of non-diffusible forms of auxins being produced in

mature leaves and moved through the phloem must be added to this concept, but direct condensation and translocation of partially condensed protein forms from the leaves may still be one of the reactions concerned. Such condensation would explain midsummer cambial growth when hormones are low or nondetectable by diffusion methods, and the effect of the removal of mature leaves, shown by

TABLE 1

CAMBIAL GROWTH AND SOLUBLE ORGANIC NITROGEN IN *POPULUS BALSAMIFERA*

SAMPLE	AGE (YEARS)	CAMBIAL GROWTH		SOLUBLE NITROGEN	
		MICRONS	DIFFERENCE	MILLIGRAMS PER 100 GRAMS	DIFFERENCE
Trunk below ring .....	8	142		110	
Trunk above ring .....	8	1036	+894*	67	-43*
Trunk below ring .....	8	141		99	
Same plus heteroauxin .....	8	302	+161*	84	-15†
Below ring, above branch .....	8	124		113	
Same but below branch .....	8	353	+229*	80	-33*
Between rings .....	8	169		113	
Same plus heteroauxin .....	8	245	+76*	99	-14*
Check in dark .....	1	44		187	
Check in light .....	1	135	+91*	143	-44*
Buds removed .....	1	0		228	
Same plus heteroauxin .....	1	54	+54*	219	-9

\* Highly significant; below 1 per cent level.

† Significant; probability of difference being due to sampling is less than 5 per cent.

PROEBSTING (11) to stop cambial growth in apple. In one of our experiments the soluble organic nitrogen of etiolated poplar leaves was reduced from 0.486 to 0.087 per cent by exposing them to normal greenhouse light for a week. Total nitrogen of the leaves decreased so that the condensed materials were apparently exported from the leaves. A sharp, although less marked, drop in the soluble nitrogen of the bark was evidence that the surplus nitrogen was not exported in unchanged form. Many other experiments could be cited which indicate that nitrogen condensation occurs in the light in mature leaves where auxin formation appears to be low or absent.

### Condensation and root formation in *Melilotus alba*

Dr. J. N. MARTIN of the Department of Botany at Iowa State College has shown in unpublished data that initiation of new branch roots in sweet clover, *Melilotus alba* Desr., is dependent upon the presence of shoots growing in the light. Roots planted in the greenhouse late in the fall of their first year, and decapitated to remove the cotyledonary buds, formed a few unbranched secondary roots, apparently from preformed root initials. If these first root tips were removed no further growth occurred unless a shoot was grafted onto the root. Reserves of carbohydrate and nitrogenous materials in the treated roots were high, and the massed meristems of preformed root

TABLE 2

SECONDARY ROOTING AND SOLUBLE ORGANIC NITROGEN IN MELILOTUS ALBA

TREATMENT	AVERAGE DRY WEIGHT ROOTLETS (MG.)	PERCENTAGE		
		DRY MATERIAL IN ROOTLETS	SUGARS IN ROOTS	SOLUBLE NITRO- GEN IN ROOTS
Control, as planted.....			11.50	0.58
Planted, decapitated.....	41	9.5	5.22	0.57
Normal, grown in dark.....	93	6.6	1.67	0.45
Normal, grown in light.....	290	8.2	1.82	0.40

tips grew at a normal or greater than normal rate. The difficulty apparently arose from the inability of the decapitated roots to form new root initials in the diffuse meristems of cortex or pericycle. Growth data from one of MARTIN's experiments with chemical analyses by the writer are shown in table 2.

Organic reserves were higher in the planted roots than the sugar percentages indicate, since there was a shift from sugar to starch in the roots held at greenhouse temperatures. Heavy shoot growth, covered to exclude light, had some stimulating effect on root growth in spite of the more rapid reduction of organic reserves. The low percentage of dry matter in the rootlets of these plants was probably the result of utilization by the tops of sugar which would otherwise have been used in differentiation of the roots. The reduction in the soluble organic nitrogen of the sprouting roots might have been assigned to utilization by the tops were it not that soluble nitrogen

percentages tend to be independent of utilization until protein reserves are depleted, a condition which was not reached in this experiment.

The striking increase in weight of rootlets on plants with their tops exposed to normal December greenhouse light was the result of the formation of more secondary roots and of profuse branching of all rootlets. In contrast, the rootlets of the darkened plants branched sparsely and those of the decapitated plants not at all. Light on leaves appears in this experiment to have been the important factor in root development. The results with etiolated plants were intermediate and resemble the results obtained with cambial growth in etiolated poplar. Soluble organic nitrogen was inversely correlated with root formation, and the explanation suggested for poplar appears to be applicable here. An increased tendency toward nitrogen condensation was accompanied by activation of a diffuse meristem, in this instance the pericycle.

#### **Condensation of nitrogenous compounds and intercalary growth in first internode of maize**

VAN OVERBEEK (12) has shown that height growth in maize, resulting almost entirely from intercalary growth, is dependent upon auxins produced by the staminate inflorescence. When the normal auxin supply is inhibited or destroyed, a dwarf type of corn is produced. INGE and LOOMIS (4) have found that intercalary growth in the first internode of germinating maize is dependent upon an uninterrupted supply of auxin from the coleoptile tip. If the coleoptile tips were irradiated, heated to 50° C., or decapitated several times at intervals of a few hours, intercalary division ceased, the embryonic region became differentiated, and the first internode lost its capacity for further growth. If the natural supply of auxin, cut off by treatment, was replaced by applying heteroauxin pastes, the treatments were ineffective in preventing growth. The massed meristems of the plumule and nodal roots were inhibited by auxins and started rapid development only after internodal growth had been arrested. The same balance between diffuse and massed meristems found in poplar appears to be involved in this correlation. Chemical analyses of plants showing internodal (diffuse meristem) growth and of



plants in which internodal growth had been stopped and plumule (massed meristem) growth stimulated, are included in table 3.

The control plants were grown in complete darkness. The lighted plants were germinated in darkness for three days and were then exposed for twenty-four hours to Mazda light at 100 f.c. This treatment is approximately the minimum illumination required to produce prompt and complete suppression of internodal growth in maize. The second lot of plants was handled in the same way except

TABLE 3  
CHANGES IN SOLUBLE NITROGEN OF MAIZE SEEDLINGS ILLUMINATED  
FOR 24 HOURS WITH MAZDA LAMPS AT 100 F.C.

TISSUE	MILLIGRAMS SOLUBLE N IN 100 GM.			
	CONTROL	LIGHTED	DIFFERENCE	"t"
After 24 hours light				
Internodes.....	154	200	+46	8.1*
Plumules and coleoptiles.....	128	152	+24	6.5*
48 hours later				
Internodes.....	166	209	+43	9.0*
Plumules and coleoptiles.....	135	145	+10	4.0†

\* Highly significant (1 per cent level).

† Significant (5 per cent level).

that the lighted plants were returned to the dark chamber and held, with the controls, for two days after being illuminated. The treated plants showed rapid plumule and nodal root growth during these two days but made no further internodal growth. In both experiments the plumule leaves did not push through the coleoptiles of the controls, and internodal growth was proceeding normally at the time the chemical samples were taken. The internode samples consisted of the entire first internode, while the plumule samples included the coleoptile and first node.

In the experiments with poplar, illumination reduced the soluble nitrogen in both the leaves and the phloem. Maize presents the apparently anomalous effect of an increase in soluble nitrogen, an increase which persisted after the plants were returned to darkness. The results obtained are considered to be due to the dominating

role of auxins in the maize correlation. The coleoptile tips of germinating grasses have been shown by many workers (3, 14) to produce or to activate growth hormones in relatively large quantities. These auxins are exported polarly downward, and are considered to be the external factor which stimulates cell division in the diffuse, intercalary meristem of the first internode. As in the previous experiments, cell division in a diffuse meristem occurred in a tissue characterized by a high degree of polymerization of organic nitrogen compounds.

When the supply of auxins from the coleoptile tips was reduced by illumination there was a rapid increase in the less condensed forms of nitrogen, and growth shifted from the diffuse to the massed meristems of the plants. The responses in three diverse types of meristematic correlations are so uniform that causal relationships appear probable.

### Discussion

The data here presented can be explained by assuming that the condensing power of diffuse meristematic tissues is low, and that rapid cell division in such tissues is possible only when their ability to condense simple organic nitrogenous compounds into protoplasm is augmented by external factors. One of these factors appears to be a continued supply of auxins. The action of natural or artificial supplies of growth substances has been demonstrated for all three of the correlations studied: cambium activity (1), root initiation (13), and intercalary growth (4, 12). Although not so well demonstrated, the possibility of the activation of diffuse meristems by direct supplies of condensed proteinaceous materials from lighted leaves must be considered. The present data show that condensation of simpler organic compounds occurs rapidly in lighted leaves. The data of MASKELL and MASON (8) indicate that proteins, as such, are exported from the leaves of cotton. On the other hand, there is much evidence that stored proteins are re-translocated only after digestion to simpler forms. It appears necessary to postulate further condensation or denaturation reactions during storage which reduces the mobility of the proteins.

During the spring, or whenever the leaf area of the plant is suddenly reduced or separated from the storage tissue by a phloem ring,

stored (or protoplasmic) proteins are digested to simple forms and sprout growth is stimulated. In this connection it is important to recall that sprout growth is stimulated also by the addition of nitrogenous fertilizers (5, 10) which result in an increase in the soluble organic nitrogen of the plant (5, 9). The production of auxins is probably increased by the added nitrogen and greater sprout growth (2), but normal balance of growth is not restored until the leaf area has been increased. During the summer, when cambial activity is greatest, the auxin level of the plant drops to a low value (1), but a balance of condensation favorable to diffuse meristems continues unless the plant is defoliated (11) or ringed above the region of cambial growth (6), in which cases cessation of cambial growth and change in nitrogen balance may be observable within twenty-four hours. All of these reactions suggest that diffuse meristems (the cambium) are stimulated by, and are able to utilize directly, intermediate forms of protein condensate supplied by the leaves. The intercalary growth of maize must be considered to be an exception to this generalization, for height growth in dwarf lines is not stimulated by light (12).

The effect of auxins upon development of diffuse meristems offers many interesting problems. Does auxin act directly in condensing nitrogenous compounds? Does it stimulate action in the meristematic cells themselves or does the condensation occur within the phloem? Is there any relation between this action of auxin and the inhibiting effect which it has on massed meristems?

Heteroauxin pastes inhibit bud development in poplar and decrease the concentration of the simpler nitrogenous compounds in the phloem. These pastes also inhibit development of marginal buds in *Kalanchoe*, but without inducing detectable changes in the nitrogenous compounds of the leaf blade tissue. These results suggest that the reaction occurs within the phloem which is inactive in detached leaves of *Kalanchoe* and which constitutes such a small percentage of the leaf that condensation which may occur in this tissue is not determinable by analysis of the entire blade. This hypothesis of condensation within the phloem is supported by the data for maize (table 3). The internodes taken for the chemical samples in the controls were 10 to 15 cm. long. The intercalary meristem occupied scarcely more than 1 per cent of this length, yet marked differences

due to light treatment were observed when the entire internode was analyzed twenty-four hours after initiating the treatment. Phloem action would be augmented in this tissue, of course, in comparison with the leaf of *Kalanchoe*, by the fact that large quantities of proteinaceous materials are being moved upward from the seed, so that actions occurring within the phloem would assume an importance entirely disproportionate to the mass of this tissue.

### Summary

1. Cambial growth in *Populus balsamifera* L. has been stimulated by (a) etiolated buds, (b) leaves in the light, and (c) applied hetero-auxin pastes. In all the experiments, shoots showing cambial activity were characterized by a reduced percentage of soluble organic nitrogen in the phloem and wood of the dividing region.

2. Initiation of new root tips in *Melilotus alba* Desr. did not occur in decapitated plants brought into the greenhouse in November. Branching was increased slightly by the presence of etiolated buds, and markedly by the presence of leaves in the light in either normal or CO<sub>2</sub> free air. Increased root formation was accompanied by decreased percentage of soluble organic nitrogen in the storage roots.

3. Elongation of the first internode of *Zea mays* L. continued for a week or longer but was stopped by illuminating the tips of the plants for twenty-four hours with a Mazda lamp at a light intensity of 100 f.c. The plants showing intercalary growth were characterized by low percentages of soluble organic nitrogen.

4. In the three species studied, diffuse meristems, cambium of poplar, pericycle of sweet clover, and intercalary meristem of maize were active when the soluble organic nitrogen content of the tissue was relatively low and inactive when it was relatively high. At high percentages of soluble organic nitrogen the massed meristems of stem and root tip were stimulated to begin or increase their development.

5. A hypothesis of condensing power is set up to explain the observed facts. According to this hypothesis the massed meristems are able to utilize simple organic forms of nitrogen and to condense these forms into protoplasm in the formation of new cells. The diffuse meristems are less effective or ineffective in condensing simple nitrogenous forms, and can develop only when supplied with

partially condensed forms of protoplasm building compounds, probably with simple albuminoid proteins.

6. The condensed forms required by diffuse meristems may be synthesized in darkness in the presence of auxins, with the phloem indicated as the seat of the reactions; or they may be formed in lighted leaves, possibly independently of known growth substances.

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# INFLUENCE OF PHOTOPERIODS UPON THE DIFFERENTIATION OF MERISTEMS AND THE BLOSSOMING OF BILOXI SOY BEANS<sup>1</sup>

H. A. BORTHWICK<sup>2</sup> AND M. W. PARKER<sup>3</sup>

(WITH EIGHT FIGURES)

## Introduction

Photoperiodism, discovered by GARNER and ALLARD (2) in 1920, has attracted the attention of many investigators who have attempted to find a physiological explanation of the results observed. GARNER (1) and MURNEEK (3) in recent papers give rather complete literature summaries. MURNEEK and GOMEZ (4) and MURNEEK (3) recognize that while many data have been accumulated descriptive of the physiological processes accompanying change of photoperiod, the causes of the reaction are still unknown. It is probable that many of the physiological studies of photoperiodism have dealt with plants in which the change in the meristems in response to photoperiod was so far advanced that the physiological conditions involved in the differentiation of flower parts were not recognized. The conditions associated with change in form of the plant may have become obscured by changes correlated with development subsequent to the earliest stages of floral initiation. It is desirable to study the physiological condition before and during the time when the shift in the type of derivative from the meristem is from strictly vegetative to floral.

## Outline of procedure

In this work a study of the occurrence and rate of development of flower primordia of the Biloxi soy bean has been made before and after the plants were subjected to various photoperiods. The objects of the experiment were: first, to determine the minimum number of short photoperiods necessary for initiation of flower buds;

<sup>1</sup> Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

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second, to compare photoperiods of various lengths as to their effectiveness in causing initiation and later development; and third, to determine certain after-effects of treatment that could not be measured until late in the development of the plants. Young seedlings were grown for about a month on a photoperiod long enough to keep them vegetative. They were then subjected for various numbers of days to a wide variety of photoperiods, many of which were short enough to cause initiation and development of flower buds. Following these treatments, the longest of which extended over a period of 10 days, the plants were placed on the natural photoperiod of about 15 hours then prevailing to continue their development. As the season progressed, the natural photoperiod gradually decreased in length to approximately 11.5 hours at the conclusion of the experiment.

About 1500 Biloxi soy bean plants were started May 28 and were grown for 40 days (until July 6) on a day length of about 17 hours. This length of photoperiod was obtained by extending the natural day from sundown until 10 P.M. by means of Mazda light. On July 6, when differential treatments were started, the plants were about 1 foot high and had five expanded compound leaves. A careful microscopic examination of all growing points on thirty-two of these plants showed that no flower primordia were present.

On this same date 1252 of these plants were selected for the experiment. One hundred were reserved as controls and were transferred directly from a 17-hour photoperiod to the natural photoperiod of about 15 hours then prevailing. The remaining 1152 plants were divided into eight series of 144 plants each. Each series was subjected to a different schedule of photoperiodic treatments (table 1).

After one day of treatment a lot of eighteen plants from each series was placed on natural photoperiod. On the second day another lot of eighteen plants was removed from each series, and so on until all the plants were used. There were eight such transfers, made at the end of 1, 2, 3, 4, 5, 6, 8; and 10 days. Thus in all there were sixty-four different experimental lots and a group of control plants.

The photoperiods for these experiments were so arranged that the midpoints of each occurred at twelve o'clock noon. This was done

so that the plants on very short photoperiods might be exposed to high light intensity. Thus the plants in any given series received on the last day of treatment a longer photoperiod than that scheduled. This final photoperiod was determined by the number of hours of daylight to which the plants were exposed after being removed from the dark chambers. The length of this final photoperiod ranged from 9 hours for the plants kept in darkness to 15 hours for the plants scheduled for a 14-hour photoperiod, as shown in table 1.

TABLE 1  
SCHEDULE OF PHOTOPERIODS OF VARIOUS SERIES  
OF TREATMENTS

	SERIES							
	A	B	C	D	E	F	G	H
Length of photoperiod on all days except last of each treatment, in hours	0	2	4	6	8	10	12	14
Length of photoperiod on last day of treatment, in hours.....	9	9	10	11	12	13	14	15

As already mentioned, the plants at the beginning of the experiment were known to have no flower primordia. Microscopic examination of control plants from day to day under natural day conditions showed that the flower primordia were first evident the last week in July. Since the scheduled differential treatments were completed on July 16, any flower primordia present at that time must have been produced in response to the treatments imposed.

### Observations

#### FLOWER BUD INITIATION

The experimental plants of the various series were examined daily for the presence of flower primordia. On the fifth day these were evident in certain lots, and between this time and the tenth day, plants from all experimental lots were examined. These observations (fig. 1) show that the stimulation brought about by two short photoperiods was sufficient to alter the course of development of the



meristems in such a way that differentiation of flower primordia resulted. Flower primordia were not visible at the end of two days of treatment, but three days later, during which time they were on natural day length, which was then greater than the critical, the

		DAYS TREATED							
		1	2	3	4	5	6	8	10
SERIES	A	—	—	—	—	—	—	—	—
	B	—	—	—	+	+	+	+	+
	C	—	—	+	+	+	+	+	+
	D	—	+	+	+	+	+	+	+
	E	—	+	+	+	+	+	+	+
	F	—	+	+	+	+	+	+	+
	G	—	+	+	+	+	+	+	+
	H	—	—	+	+	+	+	+	+

FIG. 1.—Effect of various numbers and lengths of photoperiods upon differentiation of flower primordia. Final observations made tenth day after start of differential light treatments.

beginnings of flower primordia were evident and at the end of ten days they were very conspicuous. Since two short photoperiods were sufficient to set in motion the mechanism that makes possible the differentiation of flower buds, it is obvious that fundamental physiological changes occurred during that brief period.

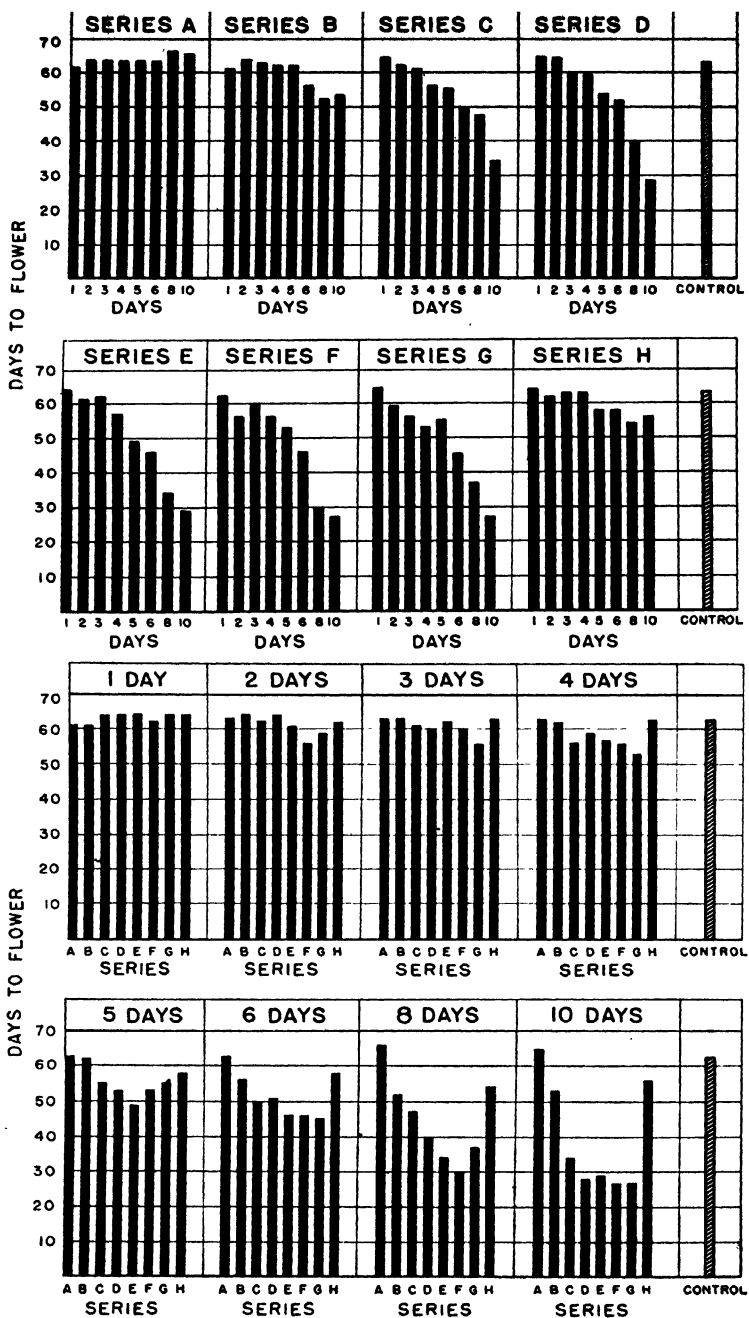
## TIME OF FLOWERING

The date of opening of the first flowers on each of eight plants in every treatment and in the controls was recorded (figs. 2, 3). It was found that all of the lots subjected to complete darkness for periods of 1 to 10 days and then placed under the conditions of natural day blossomed at about the same time as the controls (series A, fig. 2). This result would have been expected from the data shown in figure 1, for it will be recalled that no flower primordia had been differentiated during the time of treatment. The photoperiodic stimulation that finally caused them to blossom was evidently received later in the season, after they were returned to natural day. In the lots of series H (table 1), on the contrary, flower primordia were differentiated during the period of treatment but these plants also blossomed nearly as late as the controls. The plants in series D, E, F, and G (table 1), exposed only twice to their respective photoperiods, initiated flower buds, but additional days of treatment progressively hastened the opening of flowers. The first stages of flower bud initiation occur as the result of a few photoperiods that range in length from 2 to 14 hours. Subsequent development of these flower buds and their time of blossoming, however, appear to be greatly influenced by the length of the photoperiods and their number.

## STRUCTURE OF GROWING POINTS ON LONG DAY

It is evident that the structure and development of the plant, particularly its growing points, must be well known in order that the first indications of flower primordia may be recognized.

The soy bean plant growing on a photoperiod of sufficient length so that it remains vegetative develops as follows: Since the leaf primordia at the tip of the main axis and in the lateral buds are 2-ranked, a median longitudinal section cut in one plane passes through successive pairs of overlapping stipules (fig. 4*a*), and cut at right angles to this plane, passes through the midribs of the leaves and any axillary buds present (fig. 4*b*). The lateral buds as seen in figure 4*b* do not appear to develop any leaf primordia until they attain considerable size. Actually, however, leaf primordia are pres-



FIGS. 2, 3.—Effect of different numbers of photoperiods of various lengths upon time of opening of first flowers. Days to flower expressed as number of days after beginning of differential light treatments, July 6. Each bar represents mean value obtained from eight plants.

ent in the bud in the axil of the fifth leaf from the tip. To demonstrate this, longitudinal sections of the bud must be made in a plane across the leaf axil (fig. 5). The primordium in the axil of the fourth leaf from the tip of the plant as seen in this plane consists of a ridge elongated across the leaf axil (fig. 5*a*). In the next older bud a leaf primordium is seen at each end of this ridge (fig. 5*b*). These are the



FIG. 4.—Longitudinal sections through growing point of main stem of plants grown on long day: *A*, plane of section passing through successive pairs of overlapping stipules; *B*, same through midribs of leaves and axillary buds.

two prophylls which are present at the base of every vegetative branch. In the axil of each of these a bud is ultimately differentiated. The next older bud contains these primordia and also the primordium of the first compound leaf (fig. 5*c*). After the growing points of the side branches reach this stage of development they repeat the behavior of the terminal of the main stem. Each successively older bud is like the one immediately above but contains one additional compound leaf. This seems to hold true until six or eight compound leaves are formed in the bud, after which the growing point becomes apparently less active. Although there may be oc-

casional deviations from this pattern, there is remarkable uniformity so long as a photoperiod of 16 or more hours is maintained.

#### DEVELOPMENT OF GROWING POINTS UNDER SHORT PHOTOPERIOD

If the photoperiod is shortened to 14 hours or less there is a marked change in the early stages of bud differentiation. This change might easily escape notice in sections not cut in the proper plane or in dissected fresh material, unless all growing points are critically

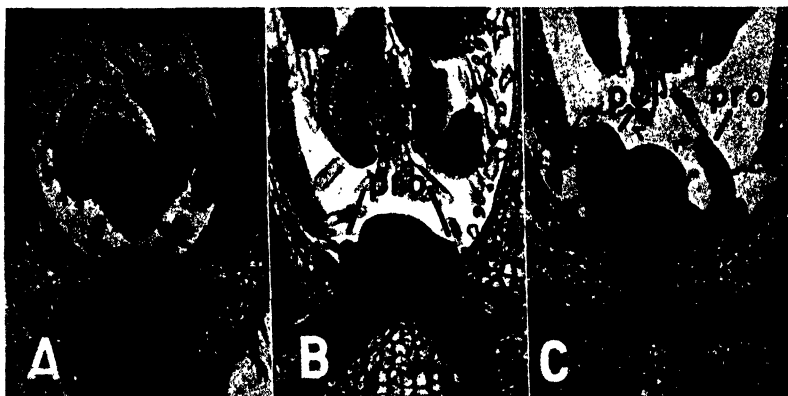


FIG. 5.—Longitudinal sections of young axillary buds of plant grown on 16-hour photoperiod. Buds A, B, and C located in axils of 4th, 5th, and 6th leaf primordia respectively from apex of main stem. *pro*, prophyll primordia; *pcl*, primordium of compound leaf.

examined. For example, in the axil of the fourth or fifth primordium from the tip the bud appears as in figure 6a, and as it grows older it goes through the stages shown in figure 6b and 6c. There are two prophyll primordia differentiated as usual, but there is almost immediately active development of the buds in their axils. The primordia of the prophylls do not seem to develop so rapidly on the flowering branches as they do on strictly vegetative ones, but the buds in their axils are relatively much more active.

The next leaf primordium above the prophylls on a flowering branch forms a small bracteal leaf, whereas it forms a large compound leaf on a vegetative branch. The difference between these structures is evident as soon as the primordia can be distinguished under the microscope, because the latter has stipules and the former

has not. In the axil of this bract there is also very early development of a bud. Usually there is a rapid succession of these bract-like primordia, the number apparently being influenced by the photoperiod and possibly other environmental factors. Instead of being 2-ranked, these primordia are spirally arranged. Each of the buds that arises in their axils gives rise directly to a single flower. In the development of the flowers a similar pair of prophylls is produced. In the flower primordium the lower part of the branch ma-

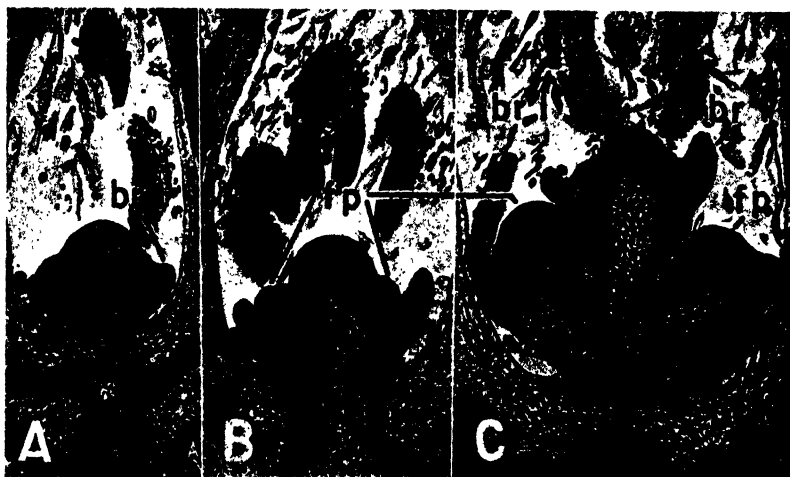


FIG. 6.—Longitudinal sections of young axillary buds of plant transferred from long to short photoperiod about 10 days before material was collected. *fp*, flower primordium; *br l*, primordium of bracteal leaf.

tures into the pedicel, however, and in its elongation the prophylls are carried outward and appear as a pair of bractlike structures, inserted on each side at the base of the receptacle. After forming the primordia of these bracts, the growing point gives rise directly to the various whorls of flower parts.

#### LOCALIZATION OF FIRST VISIBLE RESPONSE TO SHORT DAY

The first morphological change induced by short photoperiod is very definitely localized. The main axes of thirty-two plants dissected on the day the photoperiodic treatments were started averaged seventeen nodes, with relatively little variability from that mean (fig. 7). Although the axillary buds of such plants are in-

conspicuous, they actually contain the primordia of many vegetative leaves, some of which have buds in their axils.

When a plant of this type is subjected to five or six short photoperiods, the first evidence of morphological change is found in the

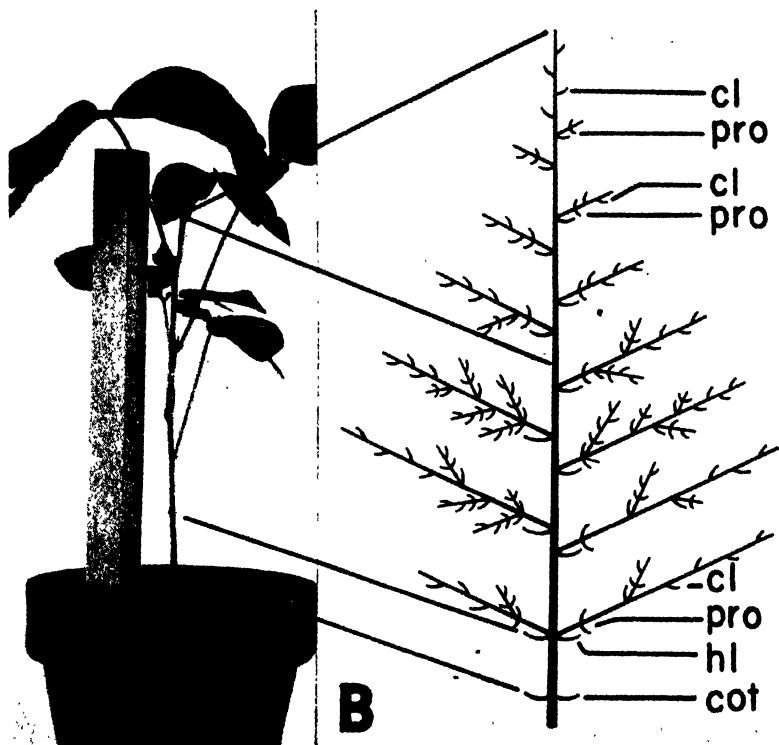


FIG. 7.—*A*, plant of approximately the same size as those used in these experiments, grown on 16-hour photoperiod. Five expanded compound leaves and total of seventeen nodes in main axis. *B*, diagram showing number and position of various structures present on plant shown in *A*. *pro*, prophyll; *cl*, compound leaf; *hl*, heart shaped leaves; *cot*, cotyledon.

bud located in the axil of the leaf primordium fourth or fifth from the tip of the main stem. Similar conditions prevail in some of the axillary buds, whereas in others there is no immediate response. This behavior of the axillary buds may be correlated with their physiological condition.

The first evidence of flower bud formation on the experimental

plants was usually found in the axil of the leaf at node fifteen, and was identified as such approximately a week after the beginning of treatment with shortened photoperiods. This bud subsequently developed into a small inflorescence consisting of a short branch bearing two reduced prophylls and two bracteal leaves, each of the latter with a single flower in its axil. The tip of the axis differentiated no additional leaves.

If the plant had not been subjected to short photoperiods this axis would have differentiated its two prophylls as usual, but the additional leaves formed would have been compound instead of bracteal. The branch would have elongated and the growing point would have continued to differentiate vegetative leaves.

At the time the differential light treatments were started, no external evidence of differentiation of a bud was apparent in the axil of the leaf primordium at node 15. In longitudinal sections through the region, however, the first stages in differentiation of the axillary growing point were evident (fig. 8). Such buds as show the earliest response to short photoperiods are in an exceedingly early stage of differentiation when the treatment is begun, and their growing points never give rise to primordia of compound leaves but produce bracteal leaves directly after the prophylls are differentiated.

#### AFTER-EFFECTS OF TREATMENT

Those growing points which are fully organized and producing primordia of compound leaves at the start of the photoperiodic treatment behave differently. The main stems of flowering soy bean plants are eventually terminated by inflorescences. In these inflorescences the flowers occur singly in the axils of bracteal leaves borne on the main stem. From this it is apparent that the growing points which during the early stages of development of the plant give rise to the primordia of compound vegetative leaves eventually give rise to primordia of several bracteal ones before they cease functioning.

At the beginning of the experiment, the plants had an average of seventeen nodes with compound leaves or their primordia located at the fifteen uppermost. The number of compound leaves present on the main axis at the end of the growing season was determined, and from this total the average number of leaves present at the



beginning of the experiment was subtracted. The remainder represented the number of compound leaves added after the beginning of



FIG. 8.—Longitudinal section through node 15 of experimental plant collected at time differential light treatments were started. First morphological changes in response to short photoperiod subsequently found at this node on experimental plants. *l*, leaf base; *s*, stem tissue; *b*, primordium of bud which makes first response to photoperiodic stimulation.

treatment (table 2). Only those photoperiodic treatments which were continued more than 6 days were effective in modifying the course of

development of the apical meristems of the primary axis, and then only in the plants in series D to G, inclusive. Even in these the growing points formed a few additional primordia of compound leaves before differentiating any bracteal ones.

Reliable data were not obtained on the behavior of the growing points of side branches. The observations indicate that they may respond to treatments of somewhat shorter duration than do the main stems.

TABLE 2

TOTAL NUMBER OF COMPOUND LEAVES ADDED AFTER BEGINNING  
OF TREATMENT WITH SHORT DAY

SERIES	NUMBER OF DAYS TREATED							
	1	2	3	4	5	6	8	10
A.....	17	18	17	17	17	15	11	.....
B.....	17	19	18	17	17	17	18	15
C.....	16	16	17	15	18	18	17	15
D.....	19	19	19	19	18	18	14	3
E.....	19	19	18	18	18	16	7	3
F.....	19	16	18	18	18	16	4	3
G.....	19	18	15	17	16	16	6	3
H.....	18	18	18	18	16	18	18	19

### Conclusions

In many studies of photoperiodism, particularly those making use of the photoperiodic after-effect, apparently little attention has been given to the condition of the terminal meristems at the beginning of the experiment and during its progress. The effectiveness of the treatment has been judged at the time of flowering by various characters which are manifest at that time. As a consequence there is no way to determine whether the treatments that were effective caused initiation of flower buds or merely accelerated development of those already present. In the treatments where no effect was noted the plants may have made no visible response whatever, or they may have initiated flower primordia that failed to reach maturity under the conditions of photoperiod.

In experiments that attempt to explain the mechanism of the photoperiodic response of plants it seems necessary to know the

complete morphological development. Time of occurrence and localization of the first changes should be known. In this work with the soy bean, localized morphological responses resulting from two short photoperiods have been observed. It seems reasonable that these changes in morphology are preceded by or associated with changes in physiology brought about during the two days' treatment. If this is true, it seems desirable for the biophysicist and biochemist to give special attention to this early period. By so doing they will be studying the tissue at the critical time before other correlated effects, which may accompany differentiation, are expressed. Such investigational procedure on the biophysical and biochemical phases of photoperiodism is being pursued in this laboratory, and the results will be reported later.

### Summary

1. Young Biloxi soy beans were grown for about a month on a long photoperiod. These long-day plants were divided into eight series, each receiving a different short photoperiod. Plants from each series were returned to natural day after 1, 2, 3, 4, 5, 6, 8, and 10 days.

2. Stimulation of two short photoperiods was sufficient to alter the course of development of the growing points in such a way that differentiation of flower primordia resulted.

3. The length of the photoperiod influenced the time of blossoming, since the plants receiving intermediate photoperiods blossomed earlier than those receiving either the extremely long or short photoperiods used in this experiment.

4. The number of days that the plants received these various treatments influenced the time of blossoming. Plants treated for 8 days blossomed earlier than those treated for only 6 days.

5. Meristems of plants growing on long day are described and compared with the meristems of those growing on short day.

6. The first visible response to short photoperiod occurred in the axil of the fourth leaf primordium from the tip of the main stem and in a similar position in certain axillary buds.

7. The region of quickest morphological response at the time the treatment is applied is an undifferentiated meristem.

8. An after-effect of photoperiodic treatment expresses itself in the total number of compound leaves produced on the main stem.

9. Treatments of less than 8 days did not suppress the addition of compound leaves.

10. Biochemical and biophysical studies which may throw light on these phenomena are in progress.

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## INDUCED PARTHENO-CARPY<sup>1</sup>

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When a flower matures, the usual thing is for pollination and subsequent fertilization, seed, and fruit production to occur, but sometimes this does not happen. At times there may be no pollination, but even then in rare instances fruits without seeds may be produced; or pollination may take place but no fertilization and no seed development, yet fruits may be formed. Such parthenocarp is not at all uncommon in some genera. Thus we have natural parthenocarp such as seedless grapes, grapefruits, oranges, cucumbers, bananas, etc.

It is difficult to determine who made the first attempts to produce parthenocarp artificially, but we know that in 1902 MASSART placed dead pollen upon the stigma of an orchid and observed a slight growth of the ovary. In 1909 FITTING (1) published his results on artificial parthenocarp in orchids. FITTING used dead and living pollen and pollen extracts. He was able to cause some slight growth in the ovary of several species of orchids when the stigma was treated with dead or foreign pollen or drops of extracts. MORITA and LAIBACH repeated and verified some of FITTING's work.

After many attempts to produce parthenocarp by cross pollination, some of which were successful and others not, YASUDA (7, 8, 9) injected aqueous extracts of pollen into the ovary, with satisfactory results. Egg-plant ovaries injected with *Petunia* pollen extract grew to be  $4.1 \times 7.3$  cm.; and in an experiment in which cucumber ovaries were injected with cucumber pollen extracts, three out of fifty ovaries grew, and at least one fruit measured  $4.3 \times 20.3$  cm., which is the size of a normal cucumber. There were no seeds. Apparently, therefore, YASUDA should be given the credit of having first artificially produced parthenocarpic fruits.

In our laboratory at Ann Arbor, we have caused normal sized

<sup>1</sup> Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

parthenocarpic fruits to develop by treating the ovaries with a lanolin paste containing the residue of a chloroform extract of pollen. Extract from the pollen of *Petunia* caused egg-plant fruits to develop to a size of  $2 \times 7$  cm., and they were still growing at the time of harvesting. *Petunia* pollen extract also caused parthenocarpy in pepper. Hubbard squash pollen extract caused cucumber ovaries to enlarge, and one large fruit and several smaller ones were produced (4).

Perhaps the most interesting work dealing with parthenocarpy is the production of fruits by treating the pistil with known chemicals, first accomplished at Ann Arbor (3). The use of known chemicals has much greater possibilities of supplying information concerning the mechanism of fruit development than has the use of pollen extract. It has been necessary, however, to show that pollen contains growth promoting substances.

In our earliest work we used indolepropionic, indoleacetic, indolebutyric, and phenylacetic acids mixed into a paste with lanolin. This paste was smeared either on the stigma or on the cut surface of the style. The first plant employed for this work was the tomato, and the first mature fruits were obtained in the late winter of 1936 with phenylacetic acid. The fruits were externally normal, but some were solid tissue with no trace of locules or seeds, while others had well developed locules but no seeds. Tomato fruits were also produced with the other compounds tried. Besides the tomato, fully developed fruits, without seeds, were also obtained in *Petunia*, *Salpiglossus*, and pepper; and egg-plant fruits were growing nicely when they had to be picked in the fall. Other plants were also used, and in some there was considerable growth, although full grown fruits were not produced.

Early in 1937, HAGEMANN (6) reported the production of parthenocarpy by treating flowers with indoleacetic acid. He secured full sized fruits, without seeds, in the gladiolus, the only plant tried in this experiment. GARDNER and MARTH (2) have introduced a new procedure which promises to be valuable. They sprayed pistillate flowers with various concentrations of the growth substances, using indoleacetic, indolepropionic, indolebutyric, and naphthalene acetic acids, in concentrations from 0.1 to 0.0001 per cent. They found the

naphthalene acetic acid to be the most effective. The plants with which they obtained success were holly and strawberry; no success was secured with apple and grape. GARDNER and MARTH also had success by watering the soil around the roots of the holly plants with 0.15 per cent indoleacetic acid solution.

For the last year we have continued this work at Michigan, and have found that injecting the material into the ovary through the pedicel is very effective in some plants like the tobacco. We have also tried the potassium salt of indoleacetic acid, and have found that in tobacco at least it is more efficient than the acid itself. This may be due, partly at least, to the fact that the salt is more soluble and a higher concentration could be used (1:500) as compared with 1:1000 of the acid.

In order to obtain additional information concerning the mechanism of fruit development, an entirely different set of experiments has been conducted (5), employing the crookneck summer squash, in which the ovules are located only at the apical end of the ovary. Although the lower end of the ovary contains no ovules, its growth rate in length is the same as for the rest of the ovary. A number of young fruits produced by pollination were cut off in such a way that only a few or no ovules were left. It was found that a considerable number of these grew, but all the fruits had some developing seeds in them.

In another experiment the ovaries from unopened flower buds were cut like the young fruits just mentioned, and the cut surface smeared with 5 per cent indolebutyric, indoleacetic, or pyrroleacetic acid. Most of the fruits treated with the first compound grew. It was very noticeable, however, that the ovaries which grew the most had some ovules present, although those ovaries from which all of the ovules had been removed grew too. This experiment tends to show that even the ovules themselves without the embryos stimulate growth in the ovaries where there has been no fertilization.

To discover whether a smaller number of seeds than normal would produce full sized fruits, experiments were performed with crookneck summer squash and pumpkin in which different amounts of the stigma were removed before pollination. The stigmatic surface was reduced by cutting off some of the lobes. In some experiments as much as five-sixths of the total stigmatic surface was removed be-

fore the pollen was applied by hand. The pollination was thus one-sided. Most of these ovaries grew into normal appearing fruits. Even though the number of seeds was much smaller than usual, their distribution was uniform. Thus reduced pollination which took place at one side of the ovary caused no change in the developing fruit, except that there were fewer seeds; but the number of seeds was sufficient for the production of a normal fruit.

From the experiments cited it is apparent that the pollen grain contains a growth promoting substance, which may be carried by the pollen tube to the ovary and cause it to grow; also the ovules may contain growth hormones, which may stimulate growth. Nevertheless, from our usual experience that unless fertilization has taken place and seed formation initiated there are no fruits formed, we are justified in believing that growth hormones are synthesized by the developing seeds, perhaps by the embryo. Sometimes, as in natural parthenocarp, the ovules and perhaps the ovary itself may produce an unusually large amount of growth hormones, and the ovary grows into a fruit without fertilization first having taken place and no seeds are formed.

That fruits can be produced artificially by supplying growth promoting substances to the ovary has now been amply demonstrated. Whether this method will have any practical application remains to be seen. GARDNER and MARTH's method looks promising for some plants. We have repeatedly tried to produce parthenocarpic fruits in summer and winter squashes, pumpkins, and watermelons with various growth promoting substances, but so far without success.

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# MERISTEMS AND FRUIT BUD FORMATION IN RELATION TO GENERAL HORTI- CULTURAL PRACTICE<sup>1</sup>

J. H. GOURLEY

## Introduction

In considering the meristematic areas at the free extremities of shoots; that is, buds or bud potentials, the horticulturist has his ultimate concern with field practices which bring about or inhibit flower formation with consequent fruit setting and development. He finds it of interest to know the causal agents or factors involved, but in the end he must manipulate the environment of the plant or apply practices which will accomplish his purposes. It should not be inferred that the horticulturist is not interested in the details, nor that he asks his colleagues in other fields to work out the solutions of his problems; the converse of this has been amply demonstrated. It does remain patent, however, that the mass of detail must be brought together and applied by the plantsman, who feels the responsibility of a great industry.

The earlier fixed concept of the genetic constitution and expression of a given variety or species of plant has given way to one of great plasticity; that is, we have come to realize, all too slowly, that if we are greatly to change one or more of the important factors of the environment, then we cannot predict with any certainty the morphological results. The unlimited potentialities of protoplasm seem literally beyond our present comprehension; some new behavior arises of which we were unaware. It should be understood that this paper presents some of the more practical applications only, and attempts to indicate some of the practices designed to initiate flower primordia which are in vogue with the commercial fruit grower, although the list is far from complete. The literature is so voluminous that little attempt has been made here to cite particular reference or authority.

<sup>1</sup> Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

### Flower bud vs. fruit bud formation

There is much confusing terminology in use among horticulturists. Since the pomologist thinks in terms of fruit production, he has long referred to flower buds as "fruit buds." True this is only a term, but the implication is erroneous. It may be useful, however, in indicating trees grown for their fruit in which there are over-wintering flower buds as contrasted with annuals, succulents, and the like. But surely a clear cut distinction should be drawn between flower bud differentiation and fruit setting. Complete flower parts may and often are laid down by the meristem and anthesis reached, and yet few or no flowers develop into fruits. The factors involved are often quite different.

Cytological studies and fruit setting experiments in various places have shown very definitely that abundant flower production is no guarantee of fruit production. For instance, there are many ornamental forms of *Prunus* which flower abundantly, but because of their unbalanced chromosome situation are non-fruitful.

### Relation of flowering to morphology

In the writer's opinion we can scarcely overstate the importance of being variety conscious in dealing with horticultural subjects. Varieties vary in their morphological detail as well as in their physiological responses to a given set of stimuli. To class all apples, or chrysanthemums, or what not, together, puts us in at least as disadvantageous a position as the plant breeder when he followed "mass selection." Then we must also learn that individuals of a given variety vary in their behavior because of differences of environment.

In referring to morphological differences of varieties, of which there are many, we may refer to the Rome Beauty apple for instance, which characteristically forms flower buds at the termini of shoots, and hence is an annual bearer. The Golden Delicious forms a large number of axillary flower buds when the trees are young, and hence has the machinery for annual flowering. Many varieties produce most of the flower buds on spurs and are much more likely to be alternate. Some, as the Livland Raspberry, develop comparatively few spurs, form few terminal or axillary flower buds on shoots, and hence are unreliable and low producers of both

blossoms and fruits. These structural situations cannot be ignored in a study of flower production of apples.

In the case of the peach and similar fruits, there is less of a problem of flower formation, owing to the occurrence of abundant lateral flower buds. It is mostly a matter of low temperature or other extraneous agencies limiting crop production.

Another morphological situation brought about as a result of some physiological response at the meristem is seen in the "ever-bearing" strawberry as contrasted with the conventional type. The latter produces flower buds during the long, warm days of summer, and shortly scapes arise, blossom, and fruit. The former forms overwintering ones during the shorter, cooler days of autumn. The buds in axils of the leaves of everbearers are mostly of flowers; hence few, if any, runners are formed. If the flower buds are removed early in the season, however, vegetative ones are formed, followed by runner formation, amounting to an increase of as much as 500 per cent in some cases, according to WALDO (8).

Yet despite these structural situations with which the horticulturist must deal, they must not be thought of as immutable, for it is this very plasticity of plant material which has given us a broader concept of the genetic expression of plants under varying environmental conditions. When we see a "carpel" terminated by an anther, a "stamen" bearing megaspores or appearing as a petal, and hear of fruits without flowers, it presents a challenge to anyone holding a static concept of plant parts.

### **Special practices affecting the apical meristem**

The horticulturist must be concerned, wittingly or unwittingly, with all factors involved in flower bud formation which can in any way be controlled. Moreover, as stated earlier, he must consider their interrelation to all practices which enter into the operation of a garden or orchard. He may wish to induce the meristems either to initiate flower primordia or to avoid it. The next step, that of fruit setting, is of equal importance but is not to be confused with the former.

But before referring to some of the practices employed by orchardists, it would be pertinent to remind ourselves that the flower buds of the deciduous fruit trees of the north are initiated the

season before they bloom. The first evidences are usually found shortly after terminal growth ceases, late in June or early in July. The duration of the period during which the floral primordia are differentiated may extend over two months or slightly longer, but the peak occurs during July. It has been shown by MAGNESS and coworkers that the period during which this can be influenced in the apple extends for about 45 days from full bloom, but the earlier the better. We are indebted to GOFF of Wisconsin for the first studies of this sort in America, at the beginning of this century, but much confirmatory work has been done by a number of others. This is mentioned because practices designed to influence this phenomenon must be timed in accordance with this knowledge.

As one surveys the field of specific practices, he is baffled as to which to cite because of their number. Among such practices or situations associated with flowering are the following:

1. Selection of variety. That variety which exhibits a strong tendency to flower regularly and freely and is otherwise desirable contributes more to a successful enterprise than all the artificial devices put together.
2. Practice of maintaining a high and uniform state of "vigor" of the trees, in which neither carbohydrates nor organic nitrogen nor nutrients are limiting. Such trees are the most regular in producing flowers and fruits, barring frosts and other hazards.
3. Girdling of the trunk or branches. Damage by fire, rodents, disease cankers, winter injury, or other injuries of this class would fall in the same category.
4. Bending of branches out of their upright position, thus contributing to an early cessation of growth extension.
5. Root pruning, which reduces the uptake of moisture, nitrogen, and other nutrients.
6. Thinning of the fruit.
7. Use of dwarfing, or semi-dwarfing, stocks.
8. Low water supply at time of initiating floral primordia.
9. Abundant leaf surface.

Some situations which are likely to limit flower formation are:

1. Excessive vegetative condition at the critical time, resulting in a carbohydrate and possibly hormone deficiency during the time of flower initiation.

2. Heavy pruning which stimulates growth extension at the expense of flower production.

3. Shading.

4. Defoliation, or injured foliage, from whatever cause.

Space will not permit a discussion of all of these factors, but a few may be mentioned in more detail.

NITROGEN AND ORCHARD CULTURE.—These two items may be treated together because available nitrogen and soil culture are closely associated in the mind of the orchardist. Nitrogen has been the element most commonly and successfully applied for the purpose of fruit production. But there is no close relationship between the amount of available nitrogen in the soil and flower bud formation. This is very different with fruit setting and development. True it has been shown by nearly all workers in this field that there is a high concentration of organic nitrogen in flowering as compared with non-flowering spurs. But frequently low-nitrogen trees bloom profusely only to lose practically all the flowers shortly after petals fall.

As one finds trees in either extreme of vegetation he sees a strong tendency toward failure of flower buds to develop, or if they develop to fail to set fruit.

In some unpublished work, the writer has found that 16-year-old Baldwin and Stayman Winesap trees, which have been treated with three times the usual amount of nitrogen, have fewer spurs forming flowers than when more moderate amounts were used. The results of similar experiments elsewhere have been so diverse that it has become apparent that it is the soil and culture that determine the fertilizer requirements, rather than the kind of fruit.

Some earlier work (1) showed that both peach and apple trees shaded with cheesecloth failed to produce flower buds as abundantly as those exposed to full sunlight, thus illustrating again that low carbohydrate-high nitrogen trees are not fruitful. Yet in all the cultural work which has been under my observation, the trees supplied with ample moisture and nitrogen were the most regular producers of flowers and fruits. The treatment to be applied depends on the starting point or the internal condition of the trees.

GIRDLING.—Ringing is the most commonly employed artificial means of bringing about flower bud formation in the apple and pear. It results in an accumulation of carbohydrates and a reduction of

nitrogen, together with other physiological phenomena, above the wound, which are associated with flower bud formation. It might be said that the effects of ringing are somewhat similar to those of root pruning, or to a sudden shift of vigorous trees to sod culture, low nitrogen supply, or more remotely to lack of pruning, or any other abrupt check in growth.

It is unnecessary to cite data to show that the removal of a ring of bark or the "scoring" of trees, or any other such injury, is likely to result in abundant flower formation, and such an observation was doubtless in part responsible for the old notion that "a tree tries to reproduce itself just before it dies." But here too, certain conditions must be met as to vigor, age, and size of tree as well as variety. For a time this old practice was unpopular, but it is now used to a limited extent in commercial orcharding, especially with "filler" trees.

**THINNING.**—Removal from a tree of a portion of the fruits before they are mature, usually when quite small, relieves competition among them, improves size, color, and quality, and influences regular bearing. For the last purpose, however, the practice has proved a great disappointment.

More recent work by the U.S. Department of Agriculture (6) has shown that earlier and heavier thinning had a most pronounced effect in increasing flower formation, even with such obstinate varieties as York Imperial and Yellow Newtown, provided the trees were vigorous and there was a high leaf to fruit ratio. In the case of Yellow Newtown, if the work was done 37 days after full bloom with seventy leaves (or more) per apple, 47 per cent of the spurs formed flower buds. If thinning was done at the same time but the leaf-fruit ratio was fifty, then only 23 per cent flowered. If the thinning was done 73 days after full bloom very few spurs flowered, regardless of number of leaves, thus emphasizing the importance of early thinning. Thus alternate bearing trees become annual ones, or if the thinning is neglected after the first year, the trees again alternate but with the crop year reversed. This is a new trend and has not as yet been put to the practical test on a commercial scale. The expense involved is a partial obstacle.

Such results would formerly have been associated entirely with conservation of food, but now the role of hormones must be con-

sidered as a second operating factor, although data on this point are lacking so far as the writer is aware.

**WATER.**—The exact role of water in this connection is not so easy to define. That water is essential for plant growth to the extent that we think of both the soil medium and the plant cells as bathed in it, is an old concept. Water is essential for flower formation of course; yet it is after a year of extreme drought that we see all sorts of perennial woody plants flowering in great profusion. This is particularly true if the water shortage is acute early in the summer. All this sounds paradoxical. If experimental evidence is examined, however, we do not find that irrigation during a dry season increases the flower crop in the ensuing year unless the trees had actually become devitalized, according to MAGNESS (5) and others. Apparently there is no close connection between amounts of water available and behavior of the meristem, as is true of inorganic nitrogen.

**PRUNING.**—Without attempting a discussion of pruning, it may be said that heavy pruning of young trees results in a marked delay in flowering and fruiting, as well as in a smaller tree, although the length of growth near the points of the cuts is increased. Practices current less than a quarter of a century ago are now practically reversed. The earlier type of pruning employed increased the ratio of leaf bud-flower bud formation, and also removed too much potential bearing surface. In other words, the whole tendency is toward lighter pruning.

#### **Factors involved in flower bud formation**

It is not possible to discuss all the practical applications in a paper of this length, but it is hoped that sufficient have been cited to indicate the diversity of the problems encountered. One or two general observations may now be made by way of summary. Throughout, two factors are prominent: (1) the need for an abundance of "healthy" green leaves, whatever they produce; and (2) a slowing or checking of growth just prior to flower bud differentiation.

Since the earliest records of man's interest in the reproductive processes of plants, there is evidence of varied speculation as to the basic factors involved. But it has been within the active period of most of us that a workable hypothesis was proposed to account for



the vast array of phenomena of reproduction. The researches and proposals relative to carbohydrate-nitrogen relationships explain more situations than any others. True they do not seem to account for all, because of our as yet incomplete knowledge, and because still other factors are involved. They have, however, given to the horticulturist for the first time a usable tool, a way of thinking of plants which can be understood and manipulated by an intelligent plantsman.

MURNEEK (7) has recently pointed out that the reverse of this situation obtained in the initiation of flower primordia in Biloxi soy beans, and it is quite likely that we cannot generalize from one species or genus to another, so far as the exact details are concerned; but in the end we are likely to come back to the necessity of an adequate food supply within the plant as a factor of major consideration.

Other more specific substances than carbohydrates and organic compounds are receiving attention as the primary causal agent of flower initiation, and interesting evidence is at hand; but in any event it is in the green leaves or other green organs that these substances find their origin. The horticulturist need not at this time quibble over nor greatly concern himself as to whether some hormone or flower promoting substance is more important than the food constituents in accomplishing the desired results. The fact still remains that flowers and their end products—fruits and seeds, are composed largely of the products of photosynthesis and of organic nitrogen complexes. It is the green leaf, unharmed by insect, disease, caustic spray solution, or other extremes of environment, that tells the story. That is, these things come within the range of the practicing horticulturist.

Of special importance is the discovery that lime-sulphur solutions reduce the rate of photosynthesis and yield of fruit in subsequent years when used at certain concentrations. A dilution of one gallon to forty of water, which was formerly a standard strength for summer application, results in reduced  $\text{CO}_2$  absorption, according to HOFFMAN (2, 3, 4), although no injury to the foliage may be apparent. When more dilute solutions are used the objection is largely obviated.

In the end, however, it is an abundance of functioning green foliage that is the key to the situation; without it other practices will avail little.

But after all, this is largely a matter of studying factors which are limiting, either inside or outside the plant. These factors may be soil nutrients, water, food supply within the plant tissues, catalysts (organic or inorganic), light, temperature. If something like an "ever-normal granary" could be maintained within the perennial woody plant, the cycles of flowering and fruiting would be less conspicuous or absent. It is because the orchardist permits exhaustion of the food reserves, is ignorant of or neglects provision for nutrient or catalyzing substances, selects unfavorable soil and site, or fails to maintain healthy foliage that there is failure of flower or fruit production.

Finally, anything that slows down or checks growth at the proper time usually results in the meristem laying down flower parts. This may come about with or without the interference of man.

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# CORRELATION OF CAMBIAL ACTIVITY WITH FLOWERING AND REGENERATION

OCRA CHRISTINE WILTON

(WITH THIRTY-SEVEN FIGURES)

## Introduction

In a previous publication, WILTON and ROBERTS<sup>1</sup> reported that a decrease in cambial activity in the higher internodes of certain herbaceous dicotyledons accompanies the production of flowers, but data showing that this condition may extend to the lower levels of the stems were not presented at that time. Information concerning the degree to which cambial activity throughout a stem is correlated with the reproductive condition is of interest as a means of eliminating the question of age of plant, which is frequently offered as an explanation of the anatomical characteristics sometimes associated with flowering. It is of further interest in its possible relation to the ability of some plants to continue or renew vegetative growth after flowering while others die at the close of the first flowering cycle.

## Material and methods

In order to determine the extent to which the cambial condition throughout the plant is correlated with the production of flowers, a study was made of cross sections of all internodes from the tips to the bases of stems of comparable flowering and non-flowering plants. The allied question of the relation of cambial activity to regeneration was investigated by examining sections from internodes both above and below second growth shoots on plants which had already passed through one flowering cycle.

From a study of more than two dozen species, the following were selected as representative for this study: *Cannabis sativa* L.; *Amaranthus retroflexus* L.; *Dianthus chinensis* L. var. *Heddewigii*; *Delphinium cultorum* Voss; *Sidalcea parviflora* Greene; *Chrysanthemum*

<sup>1</sup> WILTON, OCRA CHRISTINE, and ROBERTS, R. H., Anatomical structure of stems in relation to the production of flowers. BOT. GAZ. 98:45-64. 1936.

*morifolium* Ran. var. Lillian Doty; *Cosmos sulphureus* Cav. var. Klondike.

Material of *Cannabis* was grown in the University greenhouses during the winters of 1935-36 and 1936-37. Since this is a dioecious species, three groups of samples were taken: (1) From plants which had grown in a short-day house at 70° F., where they attained a height of more than 6 feet and were in a vigorously vegetative condition with no indication of floral buds at the time of sampling. The basal internodes were approximately five-eighths of an inch in diameter. (2) From plants which had produced pistillate flowers and at the time of sampling were bearing immature fruits. (3) From plants which had produced staminate flowers. The latter two groups were grown in a short-day environment in a house kept at approximately 60° F. For this species, the highest samples were taken from the first internode below the raceme and others from each successively lower internode to and including the hypocotyl.

The material from which samples of *Amaranthus* were taken was grown under a variety of environmental conditions in order to obtain varying degrees of reproductiveness and vegetative vigor. One group of plants was grown at a minimum temperature of 60° F. in a short-day environment. Under such conditions all of the primordia, both terminal and axillary, throughout the length of the stem differentiated as floral buds. There were no lateral branches. Such plants were termed extremely reproductive. A second group of plants was grown at 70° F., in a long-day environment, where they branched freely toward the base of the stem, required a longer time to flower, and differentiated relatively fewer floral buds than the plants grown in the cooler short-day environment. These plants were termed moderately reproductive. Plants grown in the long-day house at 60° F. were strictly vegetative, no floral structures being differentiated in the period of four months during which they were observed before sampling. Samples taken from the internode directly below the terminal inflorescence in the case of the flowering plants were designated as "first," and those from the lower internodes were numbered consecutively. The highest samples were taken from the fourth elongated internode of the non-flowering plants and also from the consecutively lower internodes. A fourth

group of *Amaranthus* plants was grown in a short-day house at 60° F. from the time of germination until a terminal inflorescence in an early stage of development could easily be recognized. The plants were then given a long-day environment although still kept at 60° F. Here development of the floral buds continued and seeds were produced, but no new floral buds were differentiated and vegetative branches were developed from the axillary buds below the terminal inflorescence. Samples were taken from internodes both above and below the new branches.

The plants of *Dianthus*, *Delphinium*, and *Sidalcea* were grown in the horticultural garden during the summer of 1936. All three species produced flowers, and at the time of sampling had developed second growth, vegetative branches. In the case of *Dianthus* the branches usually came from the second and third nodes above the ground, while the second growth branches of *Sidalcea* developed from the nodes about midway between the tips and bases. Samples of both species were taken from the internodes both above and below the new branches. The vegetative shoots of *Delphinium* came from the base of the plant just below the surface of the ground. For this reason samples were not taken below the new branches, but were taken from the basal internode of the stem and from the internode just below the inflorescence.

For the material of *Chrysanthemum* and *Cosmos*, two lots of plants were grown. One had only the light of the normal short days of autumn and in this case flowers were produced. The other lot was given an additional eight hours of artificial illumination and did not become reproductive. Photoperiod was the only environmental factor which was varied for these two species. Samples were taken from the first internode below the peduncle and from each successively lower internode of the flowering plants. Sampling was delayed until the reproductive condition was far advanced, particularly in *Cosmos* where there were numerous lateral flowering branches. The highest samples taken from the non-flowering plants were from the fourth elongated internode. The higher internodes are frequently too immature to show significant differentiation of secondary tissues.

The fresh samples were killed and fixed in formalin-acetic-alcohol.

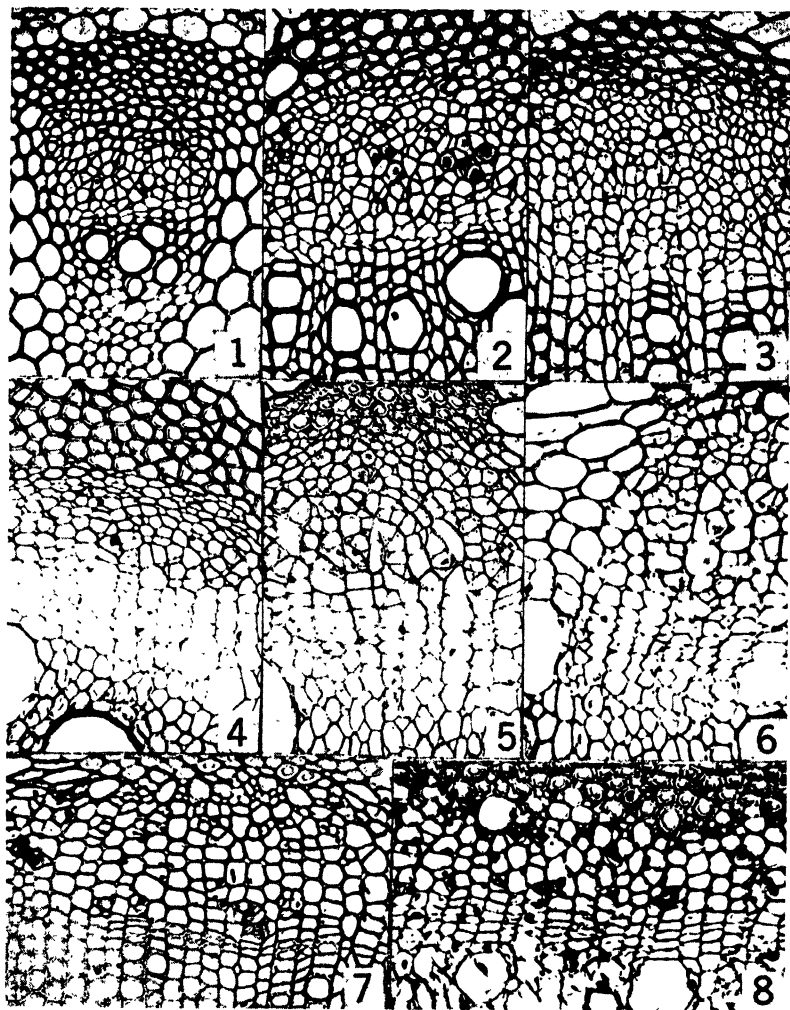
Butyl alcohol was used for dehydrating and the material was imbedded in paraffin. Sections 15  $\mu$  in thickness were made and stained with a combination of safranin and light green.

### Observations

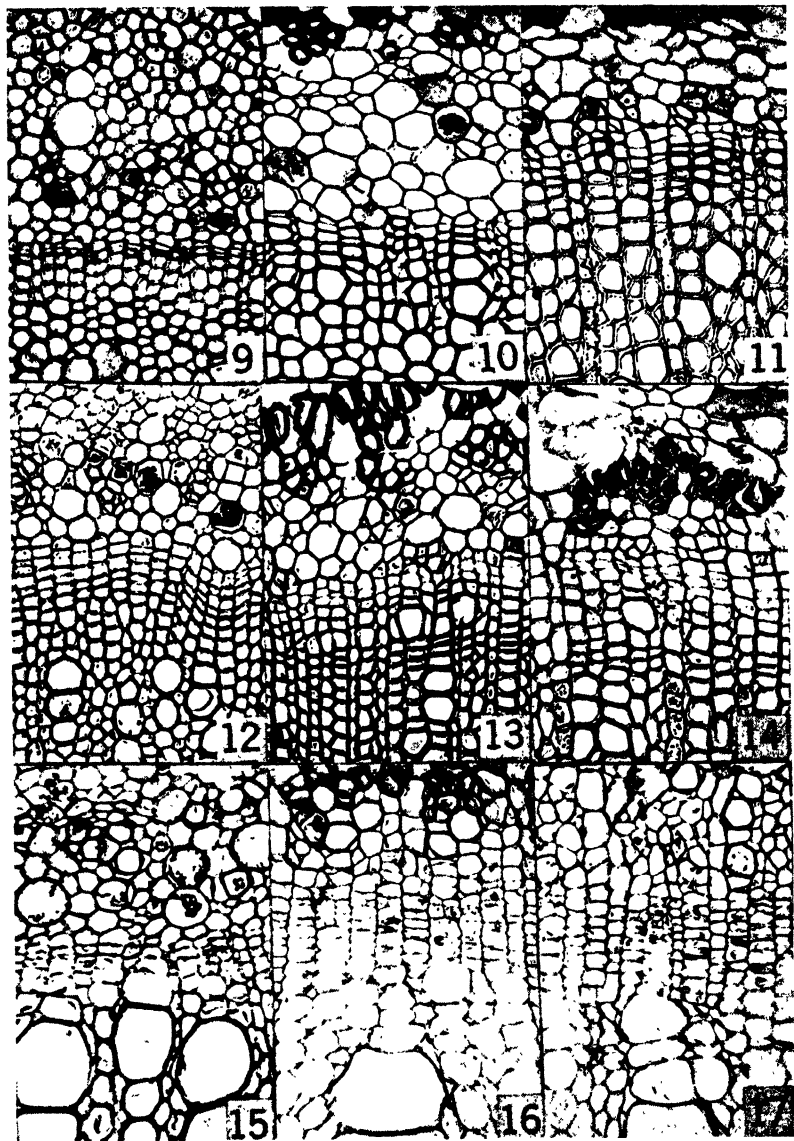
There is little or no cambial activity in any part of the stem of an abundantly flowering plant of *Cosmos* (figs. 1-3). In contrast to this condition, the non-flowering stem shows an active cambium throughout its length (figs. 4-6). The condition in *Chrysanthemum* so closely parallels that in *Cosmos* that only the basal internodes are figured (figs. 7, 8).

The slight difference in degree of maturity between the male and female plants of *Cannabis* (figs. 9-14) corresponds to their difference in vegetative vigor, which was apparent from external observation of the plants. The male plants died soon after the pollen was shed while the female plants remained green until after the seeds were mature. The stems of *Cannabis* also illustrate another characteristic which has been observed in all the species so far examined having a racemose inflorescence. Sections from the first few internodes directly below the inflorescence have relatively small cells and a weakly vegetative appearance. For this reason sections from the middle region of the stem usually give a better indication of the reproductive condition of such plants, as is illustrated by a comparison of figures 10, 13, and 16 with figures 9, 12, and 15.

The material of *Amaranthus* (figs. 18-26) also illustrates quantitative variations in degree of maturity of meristematic tissue. In this instance the variations in degree of reproductiveness were induced by differences in environmental conditions, and were evident in the external appearance of the plants. It should be noted that there is a complete absence of meristematic tissue in the stems of the extremely reproductive plants (figs. 18-20). However, a progressive decrease in thickness of the cell walls from the tip toward the base of the stem indicates a basipetal sequence in maturity corresponding to the time of cessation of cambial activity. The sharp contrast between these sections and those from the non-flowering stem (figs. 24-26) is somewhat modified by the sections from the moderately reproductive plants (figs. 21-23) which have some tissue that is at

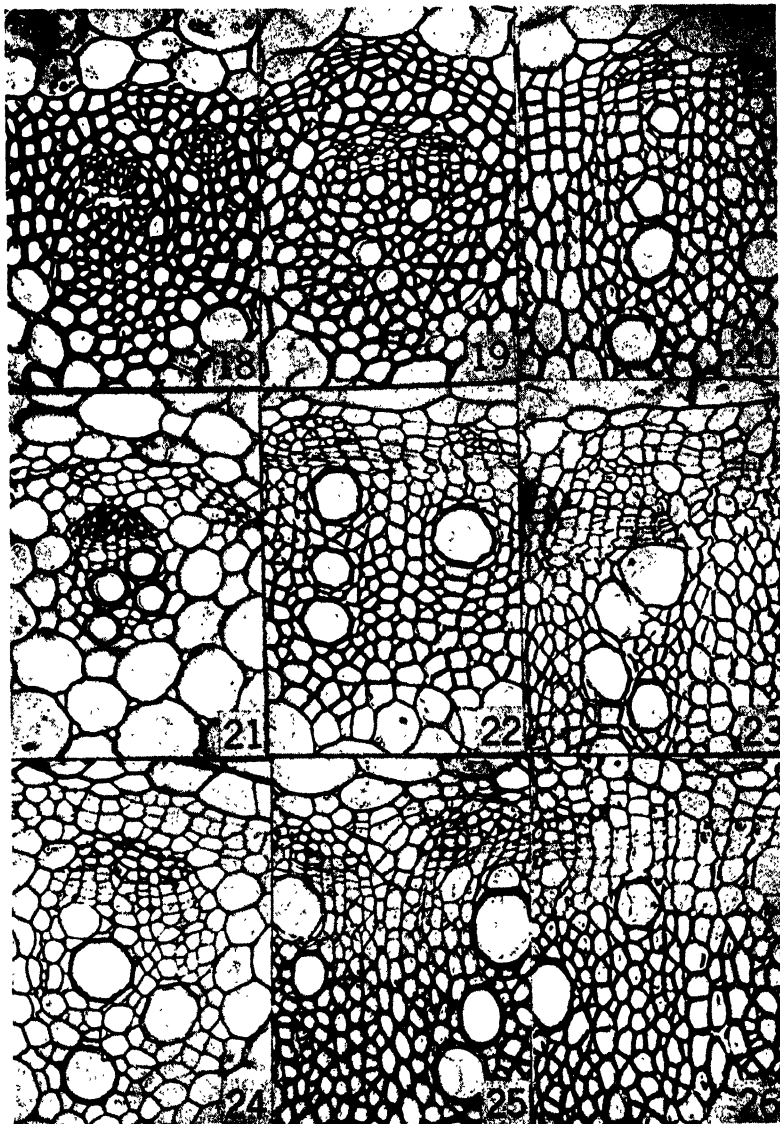


FIGS. 1-8.—*Cosmos*: Figs. 1-3, transections from second, seventh, and tenth internodes of flowering plant showing little or no cambial activity even in basal internode which was just above the hypocotyl. Figs. 4-6, comparable sections from fourth, eighth, and twelfth internodes of non-flowering plant showing active cambium throughout length of stem. *Chrysanthemum*: Fig. 7, from twenty-fourth internode of flowering stem; all elements appear to be differentiated and mature, no elements resembling cambium being present. Fig. 8, from twenty-eighth internode of non-flowering stem where there is active cambium.



FIGS. 9-17.—*Cannabis*: Figs. 9-11, sections from tip, middle, and basal regions of stem of male plant; no meristematic tissue at any level. Figs. 12-14, from stem of female plant showing a lesser degree of differentiation of cambial elements than figs. 9-11, although the only evidence of cambial activity is in the basal internode. Figs. 15-17, from non-flowering plant: 15, from fourth elongated internode, too immature to have differentiated much secondary tissue; 16 and 17, from middle and basal regions, showing active cambium.





FIGS. 18-26.—*Amaranthus*: Figs. 18-20, transections from first, seventh, and thirteenth internodes of extremely reproductive plant; complete absence of meristematic tissue and relatively large amount of mechanical tissue noted. Figs. 21-23, comparable sections from moderately reproductive plant showing more or less potentially meristematic tissue which increases progressively from tip to base. Figs. 24-26, from fourth, eighth, and twelfth internodes of non-flowering plant; amount of cambial activity markedly greater in figs. 24 and 25 than in figs. 21 and 22, where flowering condition is distinctly evident. Little difference in figs. 23 and 26.

least potentially meristematic, but with decidedly less cambial activity in the higher internodes than in the non-flowering stem.

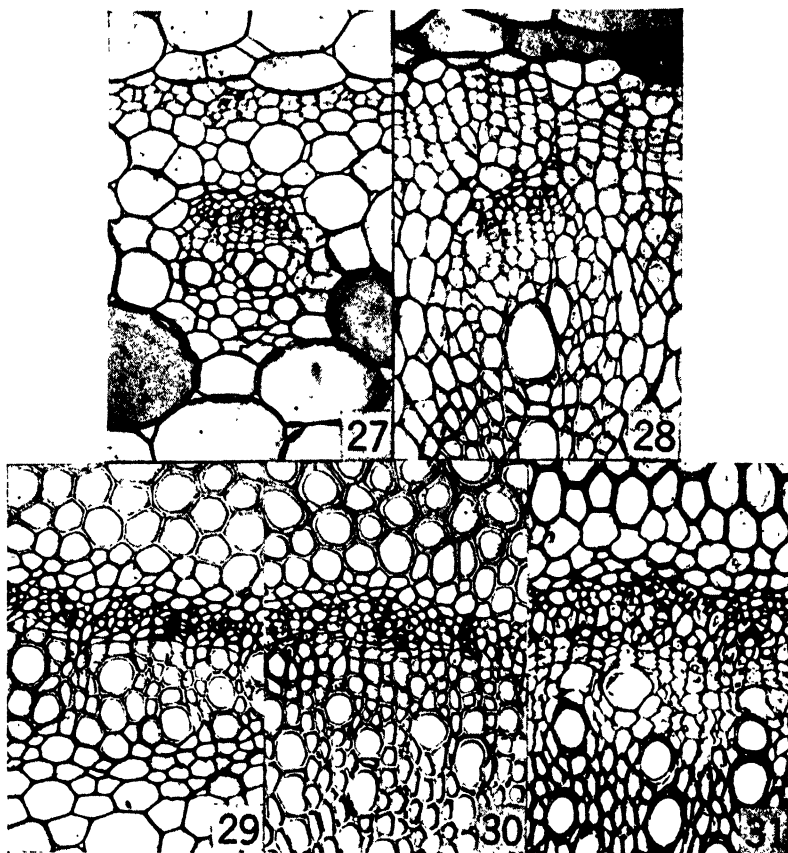
The greater degree of "maturity" of the higher internodes in contrast to the condition of the basal internodes which accompanies the differentiation of floral structures is to be interpreted as proof of the correlation of anatomical structure with flowering rather than with chronological age. Here the youngest internodes are first to become mature. Further proof is to be seen in the stems of the non-flowering plants where the opposite situation seems to prevail. These plants have a greater amount of meristematic tissue in the higher internodes than at the base of the plant.

It has been pointed out that certain species which differentiate floral buds in abundance, as do *Cosmos* and *Amaranthus* when grown at 60° F. in a short-day environment, have no meristematic tissue remaining in any part of their stems at the time of full flowering. This phenomenon suggests a possible explanation of the fact that these plants usually die at the close of one flowering cycle. It would seem that there are no elements remaining which are capable of dividing, and thus a renewal of growth is not possible even though environmental conditions may have become very favorable for vegetative development.

On the other hand, there are plants which retain some vegetative growing points while flowering either continuously or in cycles throughout a season; or after the production of flowers and fruits may lose only the terminal internodes of their branches while second growth shoots are developing at a lower level of the stem. An explanation for such habits of growth is suggested by the variations in degree of cambial activity between the tip and the base of the stems which were noted in the moderately reproductive plants, for example, *Amaranthus* grown at 70° F. in a long-day environment. It was reasoned that any part of the stem in which cambial tissue remained, either active or potential, could continue or renew vegetative growth. An attempt was made to find some definite proof for this hypothesis by an examination of the internodes, both above and below vegetative branches, which had developed after a cycle of flowering.

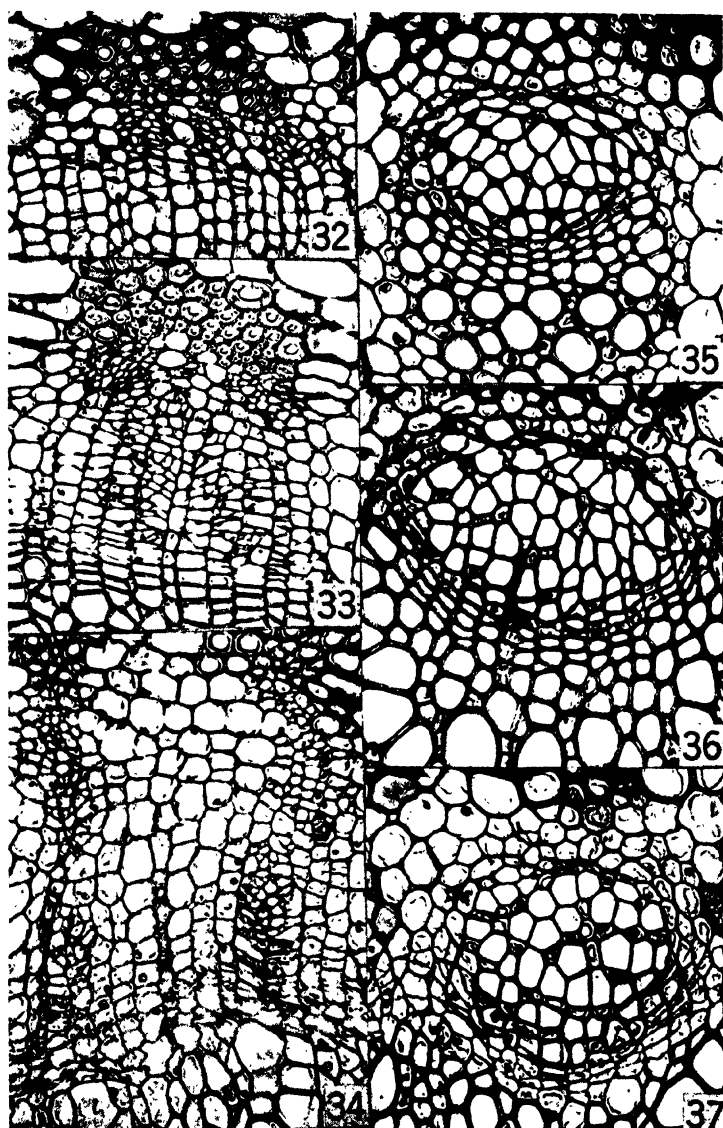
In *Amaranthus*, *Dianthus*, and *Sidalcea*, where the second growth

branches developed along the aerial stem, the internodes immediately above the highest branches have a slight amount of cambial tissue but never an active cambium (figs. 27, 30, 33). In *Dianthus*



FIGS. 27-31.—*Amaranthus*: Fig. 27, transection from third internode below inflorescence and above lateral, non-flowering branches; anatomical condition similar to fig. 23. Fig. 28, same stem below second growth, non-flowering branches. *Dianthus*: Fig. 29, below flowers; no undifferentiated tissue present. Fig. 30, same stem just above young vegetative branches; a few thin walled elements in cambial region. Fig. 31, below vegetative shoots, showing wide zone of meristematic tissue and numerous recently differentiated xylem elements.

and *Sidalcea* the higher internodes show complete maturation of the meristematic tissue (figs. 29, 32). In *Amaranthus*, where there were only three relatively short internodes between the inflorescence and



FIGS. 32-37.—*Sidalcea*: Fig. 32, transection from internode below inflorescence; no undifferentiated tissue remains. Fig. 33, from middle region of stem, above second growth branches; a few cambial elements present. Fig. 34, from basal internode below vegetative branches, showing wide cambial zone. *Delphinium*: Figs. 35, 36, transections from internode directly below inflorescence and basal internode respectively; no undifferentiated elements seen in either case. Fig. 37, characteristic structure of non-flowering stem.

the node from which two lateral vegetative branches developed, there is some tissue which is at least potentially meristematic even in the first and second internodes; but these appeared to have less vegetative vigor than the third which was photographed (fig. 27). In the case of *Delphinium* (figs. 35, 36), which did not have second growth branches along the aerial stem, the meristematic tissue was completely differentiated throughout the stem. The location of second growth branches was by no means constant for a given species, but an examination of numerous samples such as the ones figured indicated that a revival of vegetative activity is dependent upon the presence of at least a small amount of meristematic tissue. Conversely the highest second growth branches will indicate the approximate upward limit of cambial activity in the stem.

### Summary

1. If certain species of plants are allowed to reach an advanced stage of reproductiveness under favorable environmental conditions, the meristematic tissue of their stems tends to become entirely differentiated into xylem and phloem elements. This anatomical condition is a possible explanation of the death of such plants at the close of one reproductive cycle.
2. The cessation or decline of cambial activity which accompanies the production of flowers progresses from the region of the inflorescence toward the base of the plant, which it may or may not reach depending upon the degree of reproductiveness which the plant attains as measured by the relative number of primordia which differentiate floral structures.
3. Vigorously vegetative plants have an active cambium throughout the length of their stems.
4. There appears to be no correlation between the chronological age of the internode and the decrease in cambial activity in plants which are producing flowers.
5. A certain amount of at least potentially meristematic tissue seems to be necessary for a renewal of vegetative growth in stems.

## NUCLEAR SIZE IN RELATION TO MEIOSIS

J. O. BEASLEY

The majority of papers devoted to meiosis limit the observations and discussion to chromosomes. No doubt chromosomes should have the most emphasis in a consideration of meiosis, but their pairing, coiling, and movement can be more adequately explained if some attention is given the nucleus and karyolymph within which the chromosomes operate. Any study of meiosis reveals a great increase in size of first prophase meiotic nuclei over prophase nuclei in somatic cells, and it usually gives evidence of a great change in the viscosity or density of the karyolymph in the prophase stages. Although size and viscosity changes in the nucleus have frequently been observed in studies of meiosis, little seems to have been done in the way of relating them to chromosome behavior.

Various theories have been advanced to explain the chromosome behavior observed during meiosis, especially as to the cause and mechanism of chromosome pairing. DARLINGTON (1) suggests that chromosome threads normally show a strong tendency to associate in pairs. The tendency is satisfied in mitotic prophase, because the threads are double as a result of a split that occurs in the resting stage before prophase begins. Pairing in meiosis is thought to result from the precocious initiation of the prophase before the chromosomes split, which makes it necessary for chromosomes to pair in order that the tendency to be associated in pairs be satisfied.

Some observations fail to fit this scheme. In *Diptera*, METZ (4) reports paired somatic chromosomes, and other writers have reported similar phenomena, some of which were in plants. Several workers have reported double chromosomes in the leptonema stage. In a study of *Drosophila*, KOLLER (2) found salivary chromosomes to be composed of more than one strand at the time they begin to pair. In such cases chromosome pairing must be attributed to something other than a tendency of single chromosome threads to be associated in pairs. STEBBINS (8) states that at leptotene each chromosome is

composed of four chromonemata, and he points out that one difference between meiosis and mitosis is the greater length of meiotic prophase. He offers a hypothesis that the physiological condition of the nucleus in early prophase of every mitosis promotes the coming together of chromonemata, and that in meiosis the prophase is prolonged. SAX and SAX (6) point out that there is no reason to suppose that the last mitotic division differs from the others. They suggest that meiosis results from a retardation of cellular activity, as indicated by the much longer time required for meiosis than for mitosis, the longer time permitting chromosomes to uncoil completely and to pair intimately with gene by gene association. This intimate pairing was envisaged as being prevented in mitosis by the comparatively rapid prophase, which prevents the chromosomes from ever completely uncoiling.

Later it was realized that an increase in size of the nucleus, as well as the longer time required for meiosis, could be a factor in allowing chromosomes to uncoil completely and to pair.

To show the constant occurrence of increase in size of prophase meiotic nuclei over somatic nuclei, measurements were made in millimeters of the diameters of nuclei in published drawings and photomicrographs showing meiotic and somatic nuclei (table 1). It is seen that in each case there is a great increase in size of prophase meiotic nuclei over somatic nuclei. In such illustrations the somatic nuclei were probably in the resting stage. In some cases the resting stage before meiosis, or earliest meiotic (early leptotene), is given, for it is shown in table 2 that these stages differ little from somatic prophase. PAUL (5) made measurements of prophase and resting meiotic nuclei. His data show the resting nucleus to average  $7\ \mu$  in diameter, and prophase nuclei to average  $11.2\ \mu$ . The largest diameter was at synizesis, and he states "It is very tempting to conclude that the activity of the nucleus is maximum during synizesis."

In order to estimate the size of resting and prophase nuclei in mitosis and meiosis, the diameters of nuclei were measured in several parts of *Gasteria* (table 2). The measurements were made from permanent slides of sectioned material.

Prophase nuclei in root tips were  $1.48 \pm 0.149\ \mu^1$  larger in diam-

<sup>1</sup> Standard error.

eter, and they had a volume 1.44 times greater than resting nuclei in the same tissue. Somatic prophase nuclei in integuments were  $0.51 \pm 0.142 \mu$  smaller in diameter than prophase nuclei in root tips. The resting stage after the last mitotic division before meiosis aver-

TABLE 1

COMPARATIVE MEASUREMENTS OF PROPHASE MEIOTIC NUCLEI AND SOMATIC NUCLEI FROM PUBLISHED DRAWINGS AND PHOTOMICROGRAPHS IN THE AMERICAN JOURNAL OF BOTANY

AUTHOR	SPECIES	MEIOTIC (MM.)	SOMATIC (MM.)	EARLIEST MEIOTIC (MM.)	MEIOTIC SOMATIC
Pickett (1916) . . .	Arisaema	8.6	4.4	.....	1.95
Chipman (1925) . . .	Lilium superbum	50.0	.....	39.0	1.28
Sorokin (1927) . . .	Ranunculus acris	11.5	.....	7.4	1.55
Nevins (1927) . . .	Furcraea andina	4.5	2.5	.....	1.80
Cooper (1931) . . .	Lycopersicon esculentum	4.0	1.8	.....	2.22
Pastrana (1932) . . .	Begonia schmidtiana	27.0	.....	19.0	1.42
Cooper (1935) . . .	Portulaca oleracea	5.5	2.0	.....	2.75
Taylor (1931) . . .	Gasteria	17.7 $\mu^*$	.....	14.0 $\mu^*$	1.26

\* Average diameters published by Taylor.

TABLE 2

SIZE OF MEIOTIC AND MITOTIC NUCLEI IN GASTERIA

PART OF PLANT	DIAMETER ( $\mu$ )	VOLUME ( $\mu^3$ )	NUMBER
Resting in root tips . . . . .	11.38 $\pm$ 0.12	772	49
Prophase in root tips . . . . .	12.86 $\pm$ 0.09	1114	50
Prophase in integuments . . . . .	12.35 $\pm$ 0.11	986	38
Pre-meiotic prophase in anthers . . . . .	13.86 $\pm$ 0.15	1394	62
Pre-meiotic prophase in anthers* . . . . .	13.53 $\pm$ 0.13	1297	32
Resting before meiosis p.m.c.* . . . . .	11.95 $\pm$ 0.08	894	25
Beginning prophase p.m.c.* . . . . .	13.78 $\pm$ 0.10	1370	50
Prophase p.m.c. . . . .	19.05 $\pm$ 0.21	3620	33
Prophase p.m.c.* . . . . .	18.17 $\pm$ 0.19	3141	34
Prophase megaspore m.c. . . . .	19.01 $\pm$ 0.43	3597	28

\* This material was imbedded at a different time from the rest, but received approximately the same treatment.

aged  $0.57 \pm 0.147 \mu$  larger in diameter than the resting stage in root tips, and premeiotic prophase nuclei in anthers were  $1.00 \pm 0.171 \mu$  larger than prophase nuclei in root tips. These differences show that premeiotic nuclei may be slightly larger than other somatic nuclei.



Prophase (leptotene to diplotene) nuclei in pollen mother cells were  $6.22 \pm 0.209 \mu$  greater in diameter, and 3.52 times greater in volume, than the resting nuclei of pollen mother cells. The resting stage in pollen mother cells was only 1.16 times greater in volume than the resting stage of root tips, but the prophase of pollen mother cells is 3.25 times greater in volume than the prophase in root tips. Prophase nuclei of megaspore mother cells averaged 3.23 times greater in volume and  $6.15 \pm 0.439 \mu$  greater in diameter than prophase nuclei in root tips. The volume of  $894 \mu^3$  for resting pollen

TABLE 3  
SIZE OF MEIOTIC AND MITOTIC NUCLEI IN ANIMALS\*

MATERIAL	DIAMETER	VOLUME	NUMBER
Testis of mouse			
Resting somatic....	6.64	153	10
Resting meiotic....	6.25	129	10
Prophase meiotic...	10.06	533	10
Testis of grasshopper			
Resting somatic....	10.40	589	10
Prophase meiotic...	16.14	2202	10

\* I am indebted to Professor L. Hoadley for the use of slides from which these measurements were made.

mother cell nuclei and the volume of  $3141 \mu^3$  for prophase pollen mother cell nuclei correspond well with the volumes of  $950 \mu^3$  and  $3823 \mu^3$  given for *Gasteria* by TAYLOR (9). It is shown in table 3 that there is a correspondingly great increase in prophase meiotic nuclei in animals.

The increase in size of first prophase meiotic nuclei can hardly be attributed to increases in the quantity of stored food, for there is a lack of evidence of stored food in nuclei, and reproductive cells that possess large amounts of food, store it outside the nuclei. The increase in size must be a result, cause, or contributing factor in meiosis.

From most cytological preparations it appears that as the nucleus expands the karyolymph becomes more liquid, until the nucleus reaches its maximum size. The watery nature (low viscosity) of karyolymph in meiotic nuclei at zygotene is evidenced by the usual

collapsed condition of chromosome threads in preparations showing zygonema stages. From cytological observations it appears that as meiosis progresses from early zygotene, the karyolymph gradually thickens, no doubt from the diffusion of materials into it from the cytoplasm. At the end of diakinesis it seems reasonable that the osmotic values of karyolymph and cytoplasm are equal, and diffusion of substances from the cytoplasm to the karyolymph has made the two somewhat similar, which results in the disappearance of the interface or membrane that separates the nucleus and cytoplasm. KUWADA (3) found dehydration and hydration to be factors in chromosome coiling and uncoiling, and he suggests that hydration and dehydration phenomena may be related to many problems concerning mitosis and meiosis.

Doubtless the enlargement of first prophase meiotic nuclei influences chromosome behavior by increasing the space in which they are limited and by decreasing the viscosity of the karyolymph within which they move.

The relationships between length of chromosomes in different stages of mitosis and meiosis have been determined by SAX and SAX (6). They found the prophase to metaphase proportions in root tip cells to average about 3:1. The proportional length of prophase to metaphase chromosomes in meiotic cells was about 9:1. A most important point they brought out was that prophase meiotic chromosomes are from 1.4 to 2.4 times longer than prophase mitotic chromosomes. They indicated that greater length of meiotic chromosomes is the result of their being uncoiled almost completely, which permits intimate gene by gene chromosome pairing. This is impossible in somatic chromosomes, for they are always coiled. No doubt the great increase in size of first prophase meiotic nuclei is important in allowing chromosomes to expand to their full length by completely uncoiling, which is hardly possible in smaller nuclei.

Large meiotic prophase nuclei can possibly have another function, in that the greater size permits chromosomes to separate to such distances that the mutual repulsion of non-homologous chromosomes is no longer effective in keeping chromosomes in place; that is, it allows chromosomes some freedom to move independently of one

another. In smaller mitotic nuclei the repulsion of non-homologous chromosomes probably tends to keep chromosomes in the same relative position.

It is suggested (6) that there is an attraction between homologous chromosomes at all times, as indicated by occasional somatic pairing. Other evidence of a mutual attraction between homologous somatic chromosomes is paired salivary chromosomes of *Drosophila*. They are in an enlarged nucleus (2), and they remain organized as definite prophase chromosomes for a long time. It seems possible that homologous chromosomes would always associate if the karyolymph were sufficiently fluid, the prophase of sufficiently long duration, and the nucleus sufficiently large.

As has been pointed out, another difference between meiosis and mitosis is in the time required for each. The mitotic cycle has been reported to require from 30 to 340 minutes. TAYLOR (8) found in *Gasteria* that the resting period after the last premeiotic division was a long one, in which there was considerable increase in size. The total time necessary for meiosis in *Tradescantia* is estimated by SAX and EDMONDS (7) to be five or six days. Comprehensive data are unavailable regarding the comparative time required for mitosis and meiosis, but there can be little doubt that the prophase of meiosis is much longer than the prophase of mitosis.

It is seen from the preceding three points (great increase in prophase meiotic nuclear size, marked changes in viscosity of karyolymph, and comparatively great time required for meiosis) that chromosome behavior during meiosis may be more fully explained by attributing importance to other factors in addition to those considered in the current theories. In all divisions there seems to be an attraction between homologous prophase chromosomes, and the particular conditions existing in prophase of meiosis allow the chromosomes to move together and to satisfy the attraction. Perhaps it is entirely a matter of time in the prophase stage, size of nucleus, and viscosity of karyolymph that permit chromosomes to pair. Such a suggestion seems to fit most of the cytological observations, including observations that some chromosomes are split at leptotema stage, that some are paired in somatic cells, and that multiple-strand chromosomes pair in the salivary glands of *Drosophila*.

### Summary

1. Prophase mitotic nuclei are 1.44 times greater in volume than resting mitotic nuclei, while prophase meiotic nuclei are 3.52 times greater in volume than resting meiotic nuclei; and meiotic prophase nuclei are 3.25 times greater in volume than mitotic prophase nuclei.

2. Apparently the increase in meiotic nuclear size is accompanied by a decrease in viscosity of karyolymph, which results in extremely liquid karyolymph at the zygonema stage.

3. The time required for meiosis is much longer than that required for mitosis.

4. A hypothesis is advanced that the increase in volume of meiotic prophase nuclei, the decrease in viscosity of the karyolymph, and the comparatively long duration of meiosis, operate harmoniously to allow intimate gene by gene association of homologous chromosomes in the first prophase of meiosis.

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## NOTE OF AN UNUSUALLY TRANSMITTED LETHAL EFFECT IN X-RAYED SORGHUM

R. M. WHELDEN AND C. P. HASKINS

(WITH ONE FIGURE)

### Introduction

Many references occur in the literature concerning gross somatic as well as genetic effects in plants treated with x-rays. Such pioneer work as that of LOPRIORI<sup>1</sup> on *Vallisneria*, indeed, was reported very shortly after the discovery of x-rays and the design of the first gas tubes, and the work which has been reported since that time has reached enormous proportions. So far as known to the writers, however, all gross somatic modifications observed in plants arising from x-rayed seeds have occurred in the first generation; that is, in the seedlings arising from the treated seeds. The observed effects in the generation of plants arising from those which have been modified have been due either to typical genic modifications or to deficiencies in endosperm or other deformities of the seeds of plants which were themselves deficient.

It is the purpose of this paper to report a case which is somewhat different, and to the best of our knowledge, unique, involving the uniform production of deformed and lethal plants arising from seeds borne by plants, themselves of normal growth and form, which had been x-rayed as seeds, when such seeds had been rayed beyond a given critical minimum dosage. In brief, the effect observed skipped a generation, and then produced results not at all dissimilar to those ordinarily observed in a first generation of overdosed seed.

### Material and method of handling

The effect here described was observed accidentally in the course of a study of the behavior of a single-factor recessive gene for albinism under x-irradiation. The material used was a strain of sorghum developed by Dr. R. E. KARPEN at the University of Texas, and

<sup>1</sup> Schaudin in Pflüger's Arch. 77:31. 1899.

described by him some time ago.<sup>2</sup> Through his kindness our original stock was obtained from Texas in 1932, and has been bred extensively here since that time. It has been found of high uniformity, and to behave consistently as described by KARPEN.

The original purpose of the study was an investigation of modifications of the normal green-white ratio in seedlings arising from heads of sorghums, the parent plants of which had been subjected to fairly heavy dosages of x-rays as seeds. Seeds were therefore x-rayed and planted, and only those for which the applied dosage was small enough to permit the seedling to survive under field conditions were saved. These were allowed to grow to maturity in the field, were bagged before flowering, so that all daughter seeds were selfed, and were harvested. An individual record was kept of each head, and the young seedlings were grown in the laboratory on agar in Erlenmeyer flasks. It was during this procedure that the effect was noticed. The original seedlings were of course both homozygous and heterozygous for green, the homozygous whites consistently dying when the seed endosperm was exhausted. At maturity of the parent plant, however, each head was tested, and only the seeds from heterozygous green-white plants were germinated. Heterozygous and homozygous green plants are indistinguishable morphologically, as described by KARPEN.

#### Source of radiation

The source of radiation used was a Coolidge deep-therapy thick-walled tube, water-cooled and with tungsten target, operated at 200 kvp. and 30 ma., with Snook mechanical rectification, and delivering, at the point used, 120 röntgens per minute. The distance from the target to the seed was 50 cm. The seeds were mounted on a lead plate, resting on a wooden table in a lead-lined room, so that there was considerable backscatter. The shortest wave length theoretically obtainable at this voltage is about 0.07 Å., and it is probable that a considerable continuous distribution toward longer wave lengths was present. No filter was used. Use of the tube was made possible through the courtesy of the Research Laboratory and

<sup>2</sup> Genetics 16:291-308. 1931.

the Vacuum Tube Engineering Department of the General Electric Company at Schenectady.

Seeds were exposed dry, under the conditions described, for periods of 4, 8, 16, 32, 64, and 128 minutes, and planted in the open field. Seedlings from the group of longest exposure were uniformly malformed, feeble, stunted, and perished early in the field. The rest matured well. They were planted at an experimental farm on the grounds of the Schenectady plant of the General Electric Company and on the grounds of the Gray Herbarium of Harvard University in Cambridge, Massachusetts. All plants were bagged when the flowers were in young bud, "glassine" wax paper bags being used for this purpose. The seeds were harvested at maturity, those from each head being placed in a separate container labeled with the head number and exposure time of the parent seed.

The young seeds from heterozygous plants were germinated as described earlier, both sterile pure agar and agar containing nutrient medium being used. The latter was made up with Hoagland's solution and 20 per cent agar, and was adjusted to a pH of 6.5 to 7.0. Initial growth was independent of the constitution of the agar. The flasks used were of the wide-mouthed type, of one liter capacity.

### Results

Seeds from plants rayed as seeds up to 32 minutes behaved perfectly normally. The progeny of the 64 minute group, however, were malformed and defective, although the parent stock had been perfectly vigorous.

The shoots of seedlings of the 64 minute group behaved very uniformly. After reaching a few millimeters in length they became brown. Almost no development of internodes occurred, and the leaves were short, stubby, and tended to curl backward markedly, the latter effect being especially noticeable in the homozygous white seedlings (fig. 1). Otherwise there was no distinction in behavior between white and green seedlings, both browning and dying long before they had reached the heights attained by controls of either group. Control plants grew to 150-400 mm. shoot length, with an abundant root system. Early curling and browning were likewise absent in the controls of both green and white plants.

The roots showed somewhat greater variability in size and form within the 64 minute group. In most cases they were stunted in

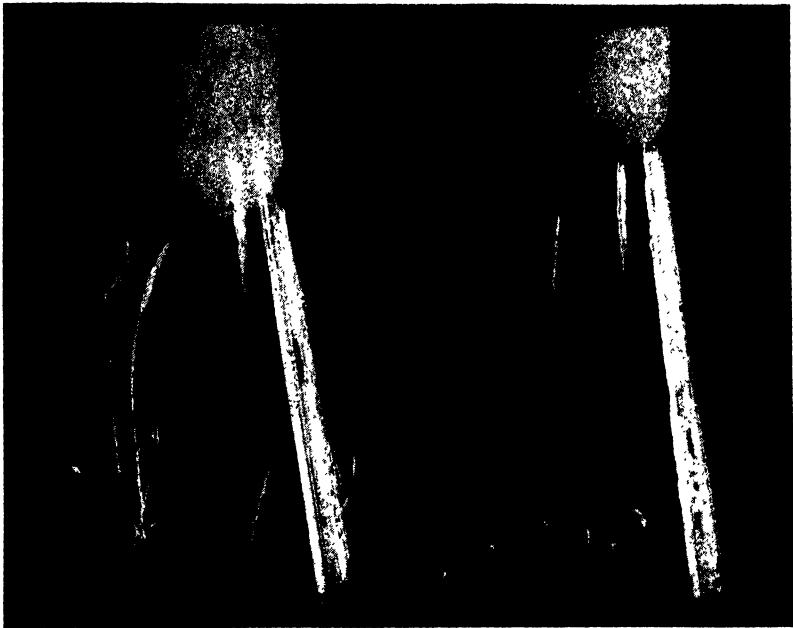


FIG. 1

TABLE 1

HEAD NUMBER	NO. PLANTS COUNTED	AVERAGE LENGTH OF SHOOT (MM.)	AVERAGE LENGTH OF ROOT (MM.)
4-64.....	49	11.8	28.9
8-64.....	134	12.9	39.7
11-64.....	20	11.6	19.9
12-64.....	113	14.9	35.2
14-64.....	74	12.1	19.0
17-64.....	123	11.3	21.1
Total.....	513	Ave. 12.6	Ave. 29.5

length and were very sparingly branched. Occasionally long root systems occurred, but they were composed of very slender rootlets. No correlation could be found between size of root and of shoot.



Microtome sections showed little difference between plants of the 64 minute group and controls, although there was a suggestion of abnormality of conducting tissue from a very early stage of development.

In all, more than 2000 plants were used in testing the effect described, and of these but two failed to show it. It seems possible that they represented seeds accidentally introduced. The data for 513 individuals are shown in table 1.

The results are clearly indicative of the uniform occurrence of what are usually considered somatic effects in the unrayed progeny of plants, themselves visibly unaffected, experiencing somewhat high dosages as seeds. Further work is in progress, and it is hoped to elucidate the nature of the phenomenon.

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# FATIGUE, SUMMATION, AND DAILY VARIATION OF IRRITABILITY IN SENSITIVE STIGMAS

HARRY J. FULLER AND JOHN H. HANLEY

(WITH TWO FIGURES)

## Introduction

Although numerous observations have been made and numerous experiments performed upon those members of the Scrophulariaceae, Bignoniaceae, Martyniaceae, and Lentibulariaceae whose stigmas exhibit turgor responses to contact and other types of stimulation, the phenomena of fatigue, summation of stimulation, and daily variation in sensitivity have been neglected. This paper presents the results of a series of experiments with reference to these subjects upon the sensitive stigmas of *Torenia fournieri*, *Catalpa speciosa*, *Tecoma radicans*, and *Mimulus luteus*. In so far as the writers have been able to determine, only one paper upon any of these subjects has thus far appeared, that of BROWN (1) who described fatigue in the stigmas of *Martynia*. The papers of NEWCOMBE (5), BURCK (2), and MACBRIDE (4) present inclusive reviews of the earlier observations upon sensitive stigmas.

## Fatigue

The stigmas of flowers of the species mentioned were stroked with a sliver of wood, and after closing of the stigmas, the time necessary for full reopening was determined. As soon as the stigmas had opened to the maximum, they were stimulated again and the time necessary for complete recovery of the unstimulated position was again noted. This repeated stimulation was continued until the stigmas remained closed, or until a recovery period of one hour was attained. The results of these experiments are presented in table 1.

It is shown in the table that with each successive stimulation the period necessary for complete recovery becomes longer. In *Torenia* and in *Tecoma* and *Catalpa* no stigmas reopened during an hour's time after the third and fourth stimulations, respectively, whereas in *Mimulus* the stigmas in fourteen of the fifteen flowers reopened after

the fifth stimulation, but did not reopen during an hour after this stimulation.

### Daily variation in sensitivity of stigmas

Stigmas of *Torenia* and *Mimulus* were stimulated by the induced current, with one electrode from the induction coil in contact with the contact-insensitive outer surface of a stigma lobe and the other

TABLE 1

RECOVERY PERIODS IN MINUTES FOLLOWING STIMULATION  
AVERAGES OF DETERMINATIONS UPON FIFTEEN  
FLOWERS OF EACH SPECIES

STIMULATION	TORENIA	MIMULUS	TECOMA	CATALPA
First.....	34	14	19	23
Second. . . . .	43	17	24 *	29
Third. . . . .	58	21	29	31
Fourth. . . . .		37	38	45
Fifth.....		55	.....	.....

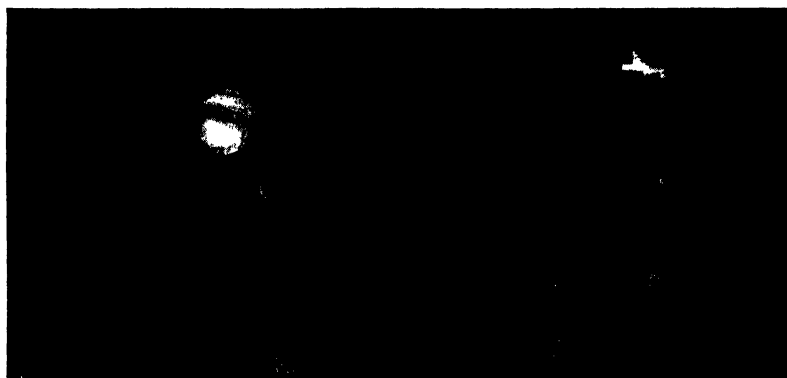


FIG. 1.—Unstimulated and stimulated stigmas of *Torenia fournieri*

electrode against the style 5 mm. below the stigma (fig. 1). The stigma was stimulated at 3-hour intervals during the day and night. The minimal stimulus required for closure at each time was taken as the index of sensitivity. Ten stigmas of each species were treated in this manner. The results are shown in figure 2, in which the value 100 represents the maximum sensitivity value, that of *Mimulus* at 3 P.M. The graph shows that sensitivity in both species rises during

the forenoon and early afternoon to a maximum between 3 and 6 P.M., after which it gradually decreases during the night to reach a minimum between 3 and 6 A.M. This variation in sensitivity corresponds roughly with that of the turgor response of *Mimosa* reported by BURGE, WICKWIRE, and FULLER (3), except that in the stigmatic responses the range of sensitivity during the 24-hour period is considerably less than that of *Mimosa*. The slight differences in some values are possibly within the experimental error, but the

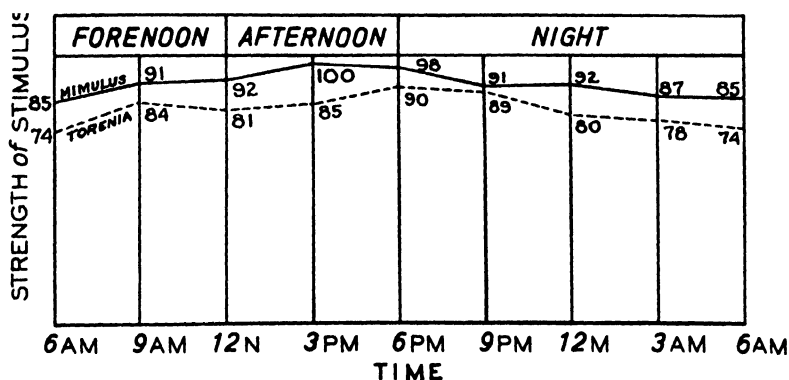


FIG. 2.—Daily variation in sensitivity of stigmas of *Mimulus* and *Torenia*

coincidence of the curves for the two species seems to indicate that sensitivity is at least slightly higher during the sunlit hours of the day than it is at night.

The graph shows also that the stigmas of *Mimulus* are slightly more sensitive than are those of *Torenia*.

### Summation of stimulation

By repeated application of the induced current in subliminal quantities, summation of stimulation with resultant closure of *Torenia* and *Mimulus* stigmas was obtained. Ten stigmas of *Torenia* and eight of *Mimulus* were treated. In all cases subliminal stimulations of less than 70 per cent of the threshold value failed to produce summation, and intervals of longer than fifteen seconds between stimulations likewise failed to produce summation. The number of applications of subliminal stimuli necessary to induce the response varied with different flowers from three to seven.

### Summary

1. Fatigue may be demonstrated in the stigmas of *Catalpa speciosa*, *Tecoma radicans*, *Mimulus luteus*, and *Torenia fournieri*.
2. The stigmas of *Mimulus luteus* and of *Torenia fournieri* are somewhat more sensitive to stimulation during the day than during the night.
3. The stigmas of these species respond as a result of a summation of stimulations of not less than 70 per cent of the threshold value presented at intervals of not more than fifteen seconds.

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# HISTOLOGICAL RESPONSES OF THREE SPECIES OF LILIUM TO INDOLEACETIC ACID<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 491

J. M. BEAL

(WITH SIXTEEN FIGURES)

The gross responses of *Lilium philippinense formosanum* and *L. harrisii* to applications of indoleacetic acid in lanolin to the cut surfaces of decapitated stems were described in a previous paper (1). The former species responded by the production of adventitious roots from approximately the surface of application to as far down as 2.5 cm. from it, while the latter produced buds in the axils of the two or three leaves nearest the surface of application, with relatively few, and in most stems no, adventitious roots. The buds also have since been shown to be strictly adventitious in origin.

In the first series tested some of the plants of *L. philippinense formosanum* had nearly mature floral buds at the time the experiments were begun. In a few cases the pedicels were cut off about 1 cm. below the receptacles and treated with the lanolin mixture. These responded in essentially the same time interval and in much the same way as did the treated stems.

A few of the floral buds were permitted to flower. The lanolin mixture was smeared over the unpollinated stigmas of some of the flowers, while in others the ovary was cut off about 5 mm. below the base of the style and the cut surface was then covered with the mixture. In both cases the ovaries enlarged somewhat, and remained green and succulent for an appreciably longer time than did pistils which were untreated and unpollinated. None of them continued to develop parthenocarpically, as was reported by GARDNER and MARTH (3) for the holly and strawberry, and by GUSTAFSON (5, 6) for various other plants. After a period of 10 to 15 days they began to shrivel and shortly afterward yellowed and died. Neither roots,

<sup>1</sup> This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

such as KRAUS, BROWN, and HAMNER (10) described on treated bean pods, nor buds were stimulated to develop on any of the treated ovaries.

Following the first experiments, which were carried out in the autumn of 1936 and the winter of 1937, it seemed advisable to test also *L. longiflorum* Thunb., a species to which *L. harrisii* Carr. is sometimes assigned as var. *eximium* Nichols. It was surprising to find that it responded in almost precisely the same manner as had *L. philippinense formosanum* in the production of adventitious roots, and that it produced no adventitious buds such as were developed by *L. harrisii*, to which it is so much more closely related. The three species used belong to the Eulirion section of the genus *Lilium*.

Approximately one hundred additional plants of *L. harrisii*, fifty of *L. philippinense formosanum*, and forty of *L. longiflorum* were used in a second series of tests made in the spring and summer of 1937. Bulbs of the respective species were potted in garden soil in 6 inch clay pots and kept under average greenhouse conditions. When the stems had reached a height of 6 to 8 inches they were decapitated just below the apical bud, which consists of a much shortened axis and a number of leaves of varying sizes and degrees of maturity. After decapitation, a single application of a 3 per cent mixture of indoleacetic acid in lanolin was spread as evenly as possible over the entire cut surface. Decapitated untreated stems were left as checks in each species and in each series.

Material for histological studies was collected at 24 hour intervals following treatment, fixed in Navashin's solution, and run up according to the butyl-alcohol paraffin method. Sections were cut at 12  $\mu$  and stained in a modified triple stain.

Because of the markedly different responses of *L. philippinense formosanum* and *L. longiflorum* on the one hand and of *L. harrisii* on the other to the lanolin mixture, the two types of responses are discussed separately.

### ***L. philippinense formosanum* and *L. longiflorum***

#### **GROSS RESPONSES TO TREATMENT**

Following decapitation and application of the lanolin mixture, only slight gross response is evident for several days. At the end of

about 48 hours a faint yellowing of the tissues adjacent to the surface of application becomes evident. During the third or fourth day the yellowing becomes more pronounced and may extend down the stem for a distance of 2 to 3 mm. The stems usually show a slight amount of swelling for a distance of 0.5 to 1 cm. below the cut surface, and the swelling may continue slowly for a period of several days, but never becomes very marked. After 10 days to 3 weeks small protuberances begin to appear from approximately the surface of application to as far down as 2.5 cm. below. The protuberances result from developing adventitious roots. In a few instances roots push up through the surface of application, but these are relatively rare as compared with the numbers which develop below this surface and which push out through the epidermis of the stem.

A small amount of callus may also develop at the surface of application in contact with the lanolin mixture, but it is usually scanty and in most examples is entirely wanting. Both callus formation and swelling of the treated stems are much less pronounced than in the bean (10) or as reported by HARRISON (7) in *Iresine*.

When the treated plants are allowed to grow for a period of 5 to 6 weeks under ordinary greenhouse conditions, the roots may push out to a length of 2 cm. or more (fig. 1B). If placed under humid conditions they elongate more rapidly, become much longer, and may remain alive for many weeks at least. Under either condition they often show a green color owing to the development of chlorophyll.

The check plants (fig. 1A) usually develop no callus on the cut surface, no swelling below it, nor do they in any case produce adventitious roots at or near the cut surface.

The treated pedicels respond in essentially the same manner as do the treated vegetative stems. They show the same type of yellowing and about the same degree of swelling, and after about the same time interval protuberances similar to those on the stems become evident.

#### HISTOLOGICAL DETAILS

The vascular bundles in the stem of *Lilium* are scattered irregularly throughout the ground meristem or fundamental parenchyma, and are of the closed collateral type. The provascular strands become recognizable a short distance back from the stem tip, and con-





FIG. 1.—*A*: decapitated untreated stem of *L. harrisii* 12 weeks after excision ( $\times \frac{1}{3}$ ). *B*: decapitated treated stem of *L. philippinense formosanum* 8 weeks after treatment, showing crown of adventitious roots (natural size). *C*: decapitated treated stem of *L. harrisii* 12 weeks after treatment; three buds (bulbils) have developed, two in axil of uppermost leaf and one in axil of second leaf ( $\times 2\frac{1}{2}$ ). Roots have developed from bulbils but not from stem.

sist of cells which are smaller and more uniform in size and with a denser cytoplasmic content than those of the fundamental parenchyma, because the cells of the latter enlarge more rapidly and differentiate earlier. Protophloem elements are differentiated in the outer third of the provascular strands and apparently become functional before protoxylem becomes evident. There is no indication of a distinguishable endodermis or pericycle associated either with the strands or with the bundles into which they develop. Whether or not a cambium is present is a matter of interpretation. In the larger and older bundles, in which a considerable amount of phloem and xylem has been formed, there is often present a group of radially flattened and tangentially elongated cells which may divide and from their derivatives additional phloem and xylem elements develop. The xylem thus derived shows annular or spiral thickenings, however, just as does the first formed metaxylem. Critical examination of older stems in longitudinal section has failed to show the presence of pitted vessels or tracheids in any bundle observed.

At the time of application of the lanolin mixture the region of the stem at the level of decapitation has the main regions well defined, although it is obvious that most of the cells have not yet reached maturity. The epidermal and outer cortical cells have comparatively dense cytoplasm and prominent nuclei (fig. 2A). Chloroplasts are present in many of the outer cortical cells. The more centrally placed vascular bundles show a number of differentiated primary phloem elements, a few protoxylem elements, and the beginning of differentiation of metaxylem. A few of the peripheral bundles are in a similar stage of development, but many of them show little differentiation other than the beginning of protophloem formation, and are still in essentially the provascular stage. The protophloem seems always to begin differentiation in advance of the protoxylem.

Twenty-four hours after application of the lanolin mixture little recognizable change is apparent except perhaps a slight increase in size of the cells of the epidermis, outer cortex, and fundamental parenchyma. As these cells enlarge their vacuoles become more pronounced, and in general their nuclei less prominent. The first pronounced responses resulting from the application of the mixture be-

come observable in stems collected 3 days after treatment (fig. 2*B*). Relatively little additional development has occurred in the vascular bundles, but some of the fundamental parenchyma cells immediately adjacent to them show an increase in the amount and density of their cytoplasm and more intensely staining nuclei. Four days after treatment the changes are even more marked (fig. 3*A*). A greater number of the cells of the fundamental parenchyma show an increased amount of cytoplasm and certain of them have begun division. A detailed view of one vascular bundle is shown in figure 3*B*. In this figure it is obvious that the responses to application of the indoleacetic acid are limited almost entirely to the cells of the fundamental parenchyma, and that few if any of the cells of the vascular strands are involved at this stage of development. In the later stages of root development, cells of the vascular bundles which have remained undifferentiated become transformed into vascular elements which serve to connect the newly formed elements of the adventitious roots to those of the original bundles of the stem. Following nuclear division a cell plate is formed immediately and wall formation quickly ensues. Thus apparently a multinucleate condition is never attained.

A moderate amount of variation in the rate of response to treatment occurs in different stems; the more highly vegetative the stem at the time of application of the mixture, the more rapid its response. Some of the stems, 5 or even 6 days after treatment, will show about the same condition as that in figure 3. Some of them develop many more roots than others. In general, however, any given lot of plants subjected to the same type of treatment exhibits a high degree of uniformity in behavior.

Following the condition just described (fig. 3), repeated mitotic divisions occur during the next few days, resulting in the production of clearly defined groups of highly meristematic cells which are usually localized in a region adjacent and centrifugal to the phloem, but which at times may almost completely surround the bundles (fig. 4*A*). This figure is from a stem collected 9 days after treatment. Infrequently the groups may lie largely at one side of the bundles. By continued divisions, and differentiation of their derivatives, these groups of cells give rise to cells which will later develop into the ad-

ventitious roots. Figure 4*B* shows the detailed structure of the young root at the right in figure 4*A*. The outlines of the original parental cell walls are often clearly recognizable even after repeated divisions have occurred within them. This is even more strikingly demonstrated in figure 5, taken from a transverse section of a stem of *L. longiflorum* 16 days after treatment, in which the sequence of events parallels almost exactly that in *L. philippinense formosanum*. The original parental cell walls persist in recognizable form for a period of several days, but sooner or later are no longer distinguishable, perhaps as a result of stretching and consequent thinning, caused by growth of the derivatives formed within.

A more advanced stage in root development is shown in figure 6. The general relation of the adventitious roots to the vascular bundles, as well as their origin from the fundamental parenchyma, is here clearly defined. So general are the mitotic divisions throughout this group of highly meristematic cells that if histogens are developed at these early stages they are not clearly indicated. As the cells continue proliferating and the whole meristematic mass increases in volume, it encroaches laterally and centrifugally upon the fundamental parenchyma which has not responded visibly to the treatment, crushing and then digesting its way through these cells and those of the outer cortex, and finally rupturing the epidermis. Later stages of root formation are shown in longitudinal sections (fig. 7*A*, *B*). These are from a stem 13 days after treatment, and show well defined roots. Evidence of an apical histogen is discernible in figure 7*B*, and cell elongation in the central core of cells foreshadows the beginning of vascular differentiation.

Following the general blocking out of the root, xylem elements are differentiated near the base and periphery of the inner core of cells. The first xylem differentiated appears always to be tracheids, which connect the vascular systems of root and stem. Simultaneously phloem is differentiated in the same general region but on alternate radii, and it also forms a continuous system between root and stem. New tissues are added apically, and as growth continues the young root pushes out through the covering layers of the outer cortex (fig. 8). The main regions are clearly distinguishable, and through continued growth and under favorable conditions the roots quickly

emerge from the stem and mature in much the same way as do secondary roots. The induced roots appear normal in all respects.

The treated pedicels, while generally responding to the treatment like the stems of *L. philippinense formosanum*, show one rather marked difference. Nearly all, and in some cases all, of the vascular bundles are associated with the production of roots (fig. 9). These are developed in all respects in the same way as are those from the stems of the same species.

### ***Lilium harrisii***

#### **GROSS RESPONSES TO TREATMENT**

Yellowing and slight swelling of the stems immediately below the surface of application occur in this species much as in the two previously discussed, but there is a greater range of variation in response in *L. harrisii*. A larger amount of callus may occasionally develop over the surface of application, and in some stems may even develop in the axils of the upper one or two leaves. A few of the treated plants develop adventitious roots, but these almost invariably come from the callus tissue rather than from the fundamental parenchyma about the vascular bundles, as occurs in the other two species described. Large tumors have not been produced by any of the treated stems.

By far the most striking change is the development of the buds, not visible at the time of treatment, in the axils of the upper two or three leaves (fig. 1C). In a very few cases buds develop from the callus tissue on the cut surface of the stem, but these cases may possibly be associated with cells in the leaf axil where the plane of cutting the stem passed through or partly through this region. In the great majority of cases observed the buds arise directly in the leaf axils without the formation of callus and upon stems from which no adventitious roots arise. The number of buds developed per plant varies from one to as many as four. In one instance three were observed in a single leaf axil, but one or two is the usual number.

The buds arise near the side of the leaf attachment as frequently as in the central or strictly axillary position. When two are present in an axil, one being strictly axillary and the other accessory in posi-

tion, they may be equal in size or either may outgrow the other. Apparently the one first formed will grow the more rapidly.

Two to 3 weeks after becoming evident, the buds begin to assume a bulbous form. At this time, and subsequently, they resemble closely the bulbils which form naturally near the bases of the untreated plants of this species. Figure 1C shows a plant about 3 months after treatment, which has developed four bulbils. The largest of these has leaves approximately  $1\frac{1}{2}$  inches in length, and all the bulbils have developed adventitious roots at their bases. Bulbils which have developed to the size of the largest one on this plant can be removed from the parent plant and grown independently in sand or soil.

None of the check plants produced either buds or roots over a period of about 4 months (fig. 1A), after which time the stems began to die.

#### HISTOLOGICAL DETAILS

The structure of the normal stem of *L. harrisii* closely parallels that of the other two species. Both longitudinal and transverse sections have been cut, but since the clearest details of bud development have been shown by the longitudinal sections, these have been used exclusively for the photomicrographs of this species.

A median longitudinal section from a decapitated but untreated stem, typical of the stage of development at the level and time of application of the mixture, is shown in figure 10A. It is obvious that most of its cells have not reached maturity, although relatively few additional cell divisions would probably occur in this region under the usual conditions of growth. The cells contain comparatively large vacuoles with a rather scanty amount of cytoplasm surrounding them. The cell walls are thin.

Twenty-four hours after application of the mixture definite changes are observable (fig. 10B). The epidermal cells immediately above the leaf axil have enlarged, chiefly in the radial plane, while the outer cortical cells opposite them on the stem side as well as below the axil have also enlarged. An increase in density of their cytoplasm is evident, together with larger and more deeply staining nuclei. The cell walls throughout the entire section appear somewhat

thicker. The first observable changes in the treated stems are represented by this group of cells, which marks the general region of initial response at which the induced buds will appear later.

Little additional change, other than continued enlargement of cells in the region of the leaf axils and increase in the amount and density of their cytoplasm, is noticeable at the end of 3 days following application of the mixture (fig. 10C). A few nuclei show early prophase stages in mitosis, but as yet none has divided. Four days after treatment, some cells of both the epidermis and outer cortex have undergone division; although all the cells in this portion of the stem have continued enlargement, only those in the immediate vicinity of the leaf axil have started dividing (fig. 10D).

At the end of 6 days following treatment a larger number of the outer cortical cells have undergone division, and in many of them their derivatives in turn have divided (fig. 11A). The comparatively thick walls of the parental cells in this figure, as well as in the next, stand out conspicuously in contrast to the thin and scarcely distinguishable ones between the daughter nuclei. Just as in the other two species, they usually remain clearly evident for a period of several days, and may be recognized up to the eleventh or twelfth day following treatment; finally they are no longer discernible.

Subsequent changes proceed fairly rapidly. A stem collected 7 days after treatment shows a larger area of meristematic activity (fig. 11B). As a result of repeated divisions and subsequent growth of the derivatives, a hump of tissue has developed in the axil. The epidermal cells show increased radial elongation, but comparatively few divisions have occurred as yet in its cells. Eight days after treatment more of the epidermal cells have divided and the hump has become more pronounced (fig. 12A). Through further divisions and enlargement of the derivatives the condition shown in figure 12B is reached in about 3 days more, or 11 days after treatment. In the more actively growing portion of the embryonic bud it is no longer possible to distinguish the outlines of the original parental cell walls, although they are still visible in the center of the figure where growth has proceeded more slowly. A later stage in development is shown in figure 13, which is from a stem 14 days after treatment. The hump of cells seen in figure 12A and B has differentiated into an axillary bud

consisting of a growing point and two leaf primordia. The beginning of vascular differentiation is evident from a point near the base of the bud for a distance downward in the tissue of the stem. Pro-vascular strands appear to be initiated a short distance back from the growing point of the buds, in essentially the same fashion as in the ordinary vegetative stems of *Lilium*, and in the same scattered arrangement. Differentiation of primary phloem and xylem proceeds in the same way.

Two more figures are shown to illustrate later stages in bud development. Both are from a stem 39 days after treatment, but figure 14 is from a somewhat less advanced stage of development than that of figure 15. Both show considerable evidence of vascularization, and from figure 15 it is only a short step to the formation of a bulbil such as those illustrated in figure 1C.



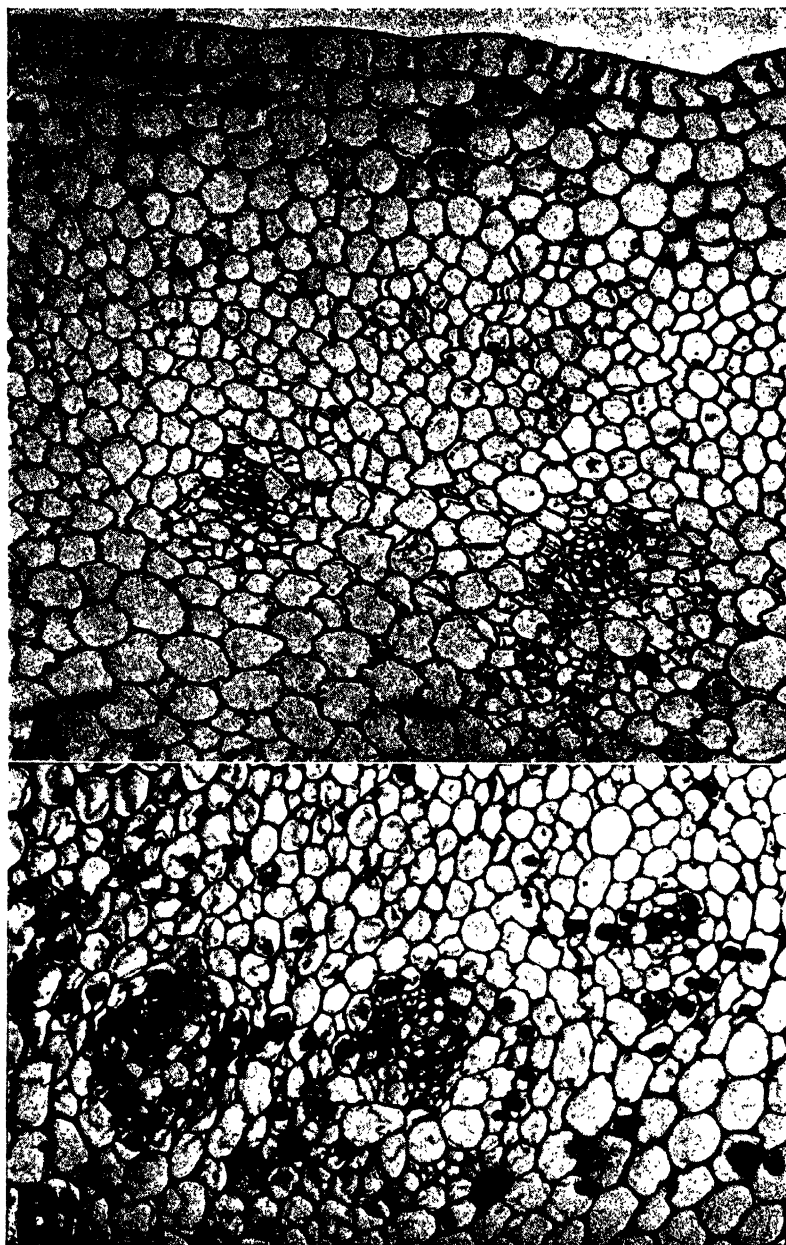


FIG. 2.—*L. philippinense formosanum*. A: transection about 2 mm. below treated surface at time of treatment; cells contain large vacuoles and inconspicuous nuclei. B: 3 days after treatment; fundamental parenchyma about vascular bundles shows denser cytoplasm and more deeply staining nuclei.

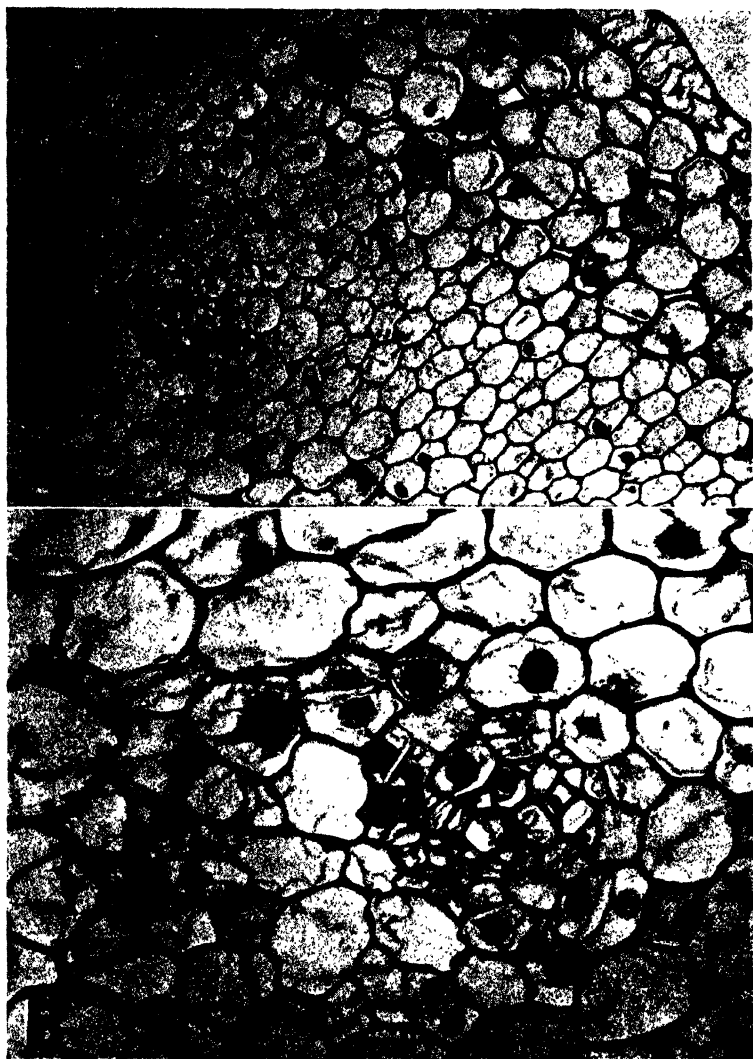


FIG. 3.—*L. philippinense formosanum* 4 days after treatment, about 4 mm. below treated surface. *A*: beginning of meristematic activity in fundamental parenchyma. *B*: details of same bundle enlarged. Phloem differentiated; primary xylem cells centripetal to those enlarging. Several fundamental parenchyma cells divided; cell walls separate their daughter nuclei. One cell shows cell plate formation.

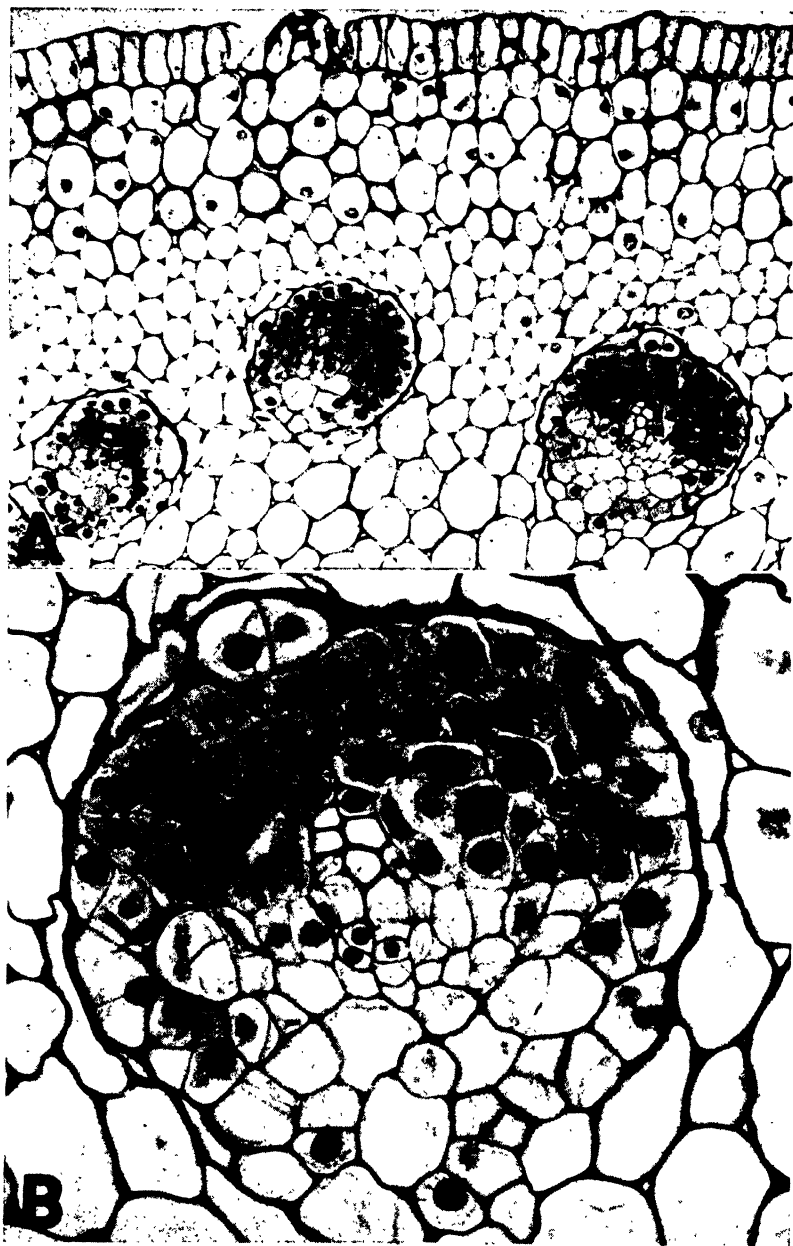


FIG. 4.—*L. philippinense formosanum* 9 days after treatment, section about 4 mm. below treated surface. A: marked increase of meristematic activity in fundamental parenchyma; xylem not fully matured. B: enlarged view of young root at right in A.



FIG. 5.—*L. longiflorum*, transection of stem 16 days after treatment, about 3 mm. below treated surface. Young root slightly more advanced than in fig. 4, showing original parental cell walls about their numerous derivatives. Cells of fundamental parenchyma which have not divided are being crushed.

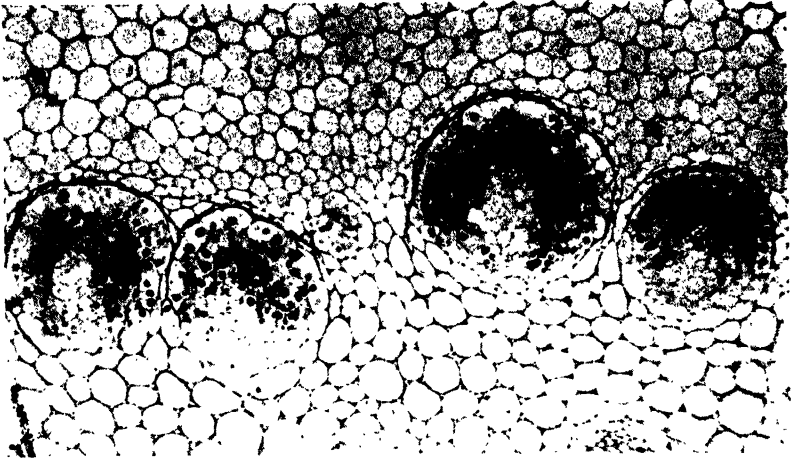


FIG. 6.—*L. philippinense formosanum*, more advanced stages than fig. 4. Meristematic activity marked in four young roots; crushing of fundamental parenchyma about growing roots.



FIG. 7.—*L. philippinense formosanum*. A: 13 days after treatment, longitudinal section of stem and median longitudinal section of adventitious root. B: same age as A, more advanced. Apical histogen and regions of root differentiated.



FIG. 8.—*L. philippinense formosanum* 21 days after treatment. Longitudinal section of stem and median longitudinal section of basal portion of adventitious root showing connection of xylem of root and of vascular bundle of stem.

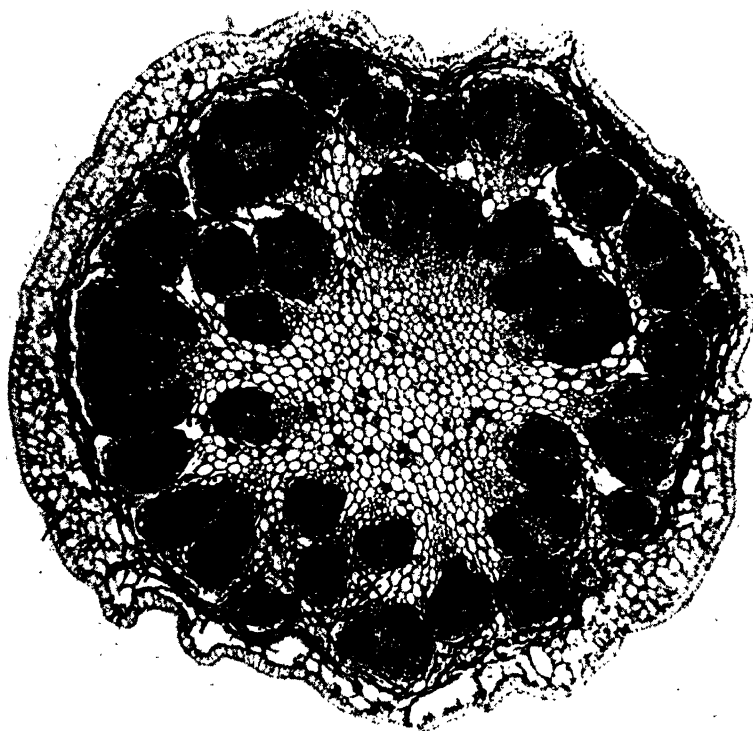


FIG. 9.—*L. philippinense formosanum*, transection of pedicel 21 days after treatment. Roots developing in association with all vascular bundles. Much crushing and disorganization of fundamental parenchyma and outer cortex.



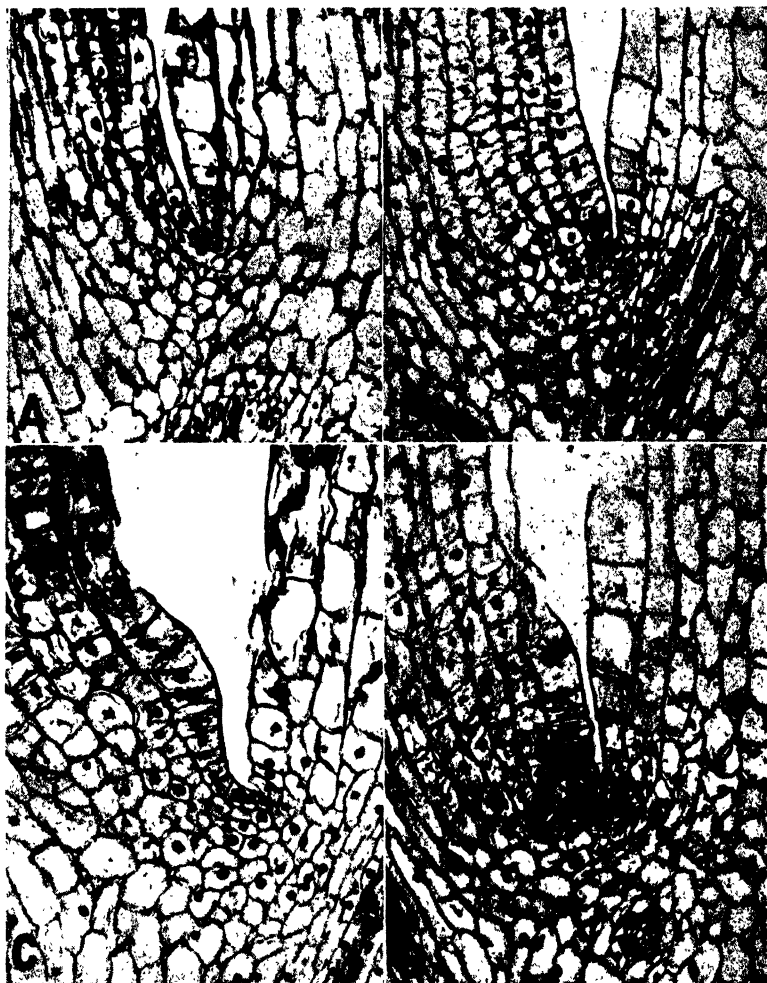


FIG. 10.—*L. harrisii*, longitudinal section through first leaf axil below surface of application. *A*: untreated check at time of treatment. *B*: 24 hours after treatment; epidermal cells above axil radially elongated; outer cortical cells near axil unchanged. *C*: 3 days after treatment; epidermal cells more elongated radially; outer cortical cells near axil with denser cytoplasm and prominent nuclei. *D*: 4 days after treatment; one epidermal cell is dividing and a few cortical cells have completed divisions.

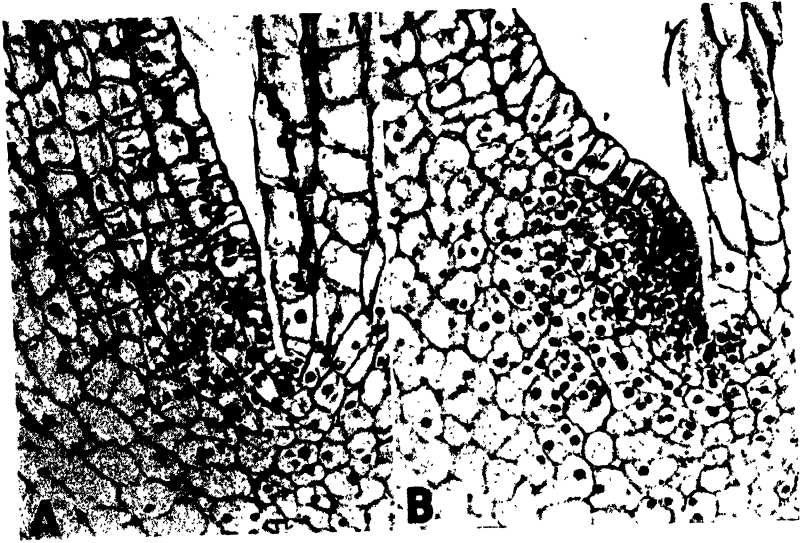


FIG. 11.—*L. harrisii*. *A*: 6 days after treatment. Many of outer cortical cells near axil divided, and their derivatives in some again divided. *B*: 7 days after treatment. Marked meristematic activity evident in fairly extensive area of cortical cells on stem side and below axil. Original parental cell walls evident, in some cases inclosing a number of derivative cells.

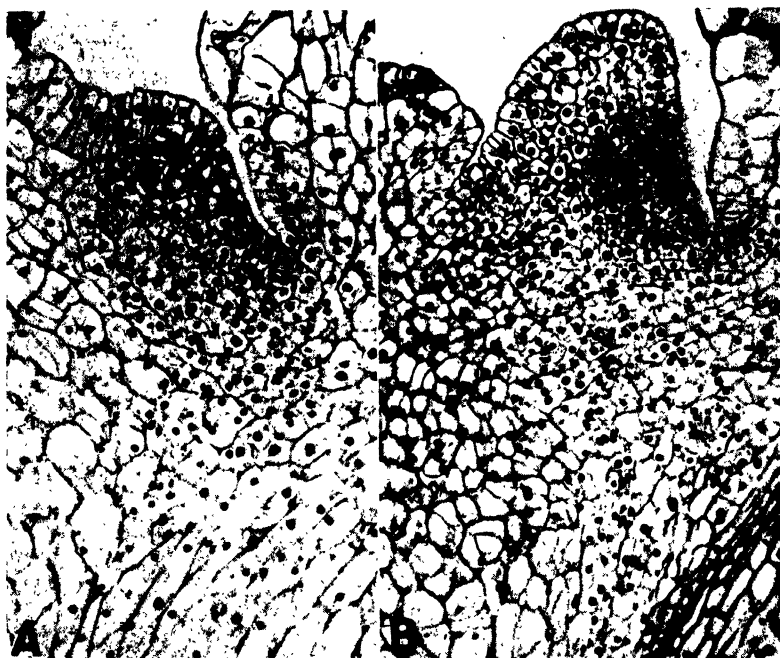


FIG. 12.—*L. harrisii*. *A*: 8 days after treatment; area of meristematic activity extended and growth of derivatives resulting in hump in axil. *B*: 11 days after treatment; hump much more pronounced. Original parental cell walls no longer recognizable in upper portion of embryonic bud, but still evident near center of figure.

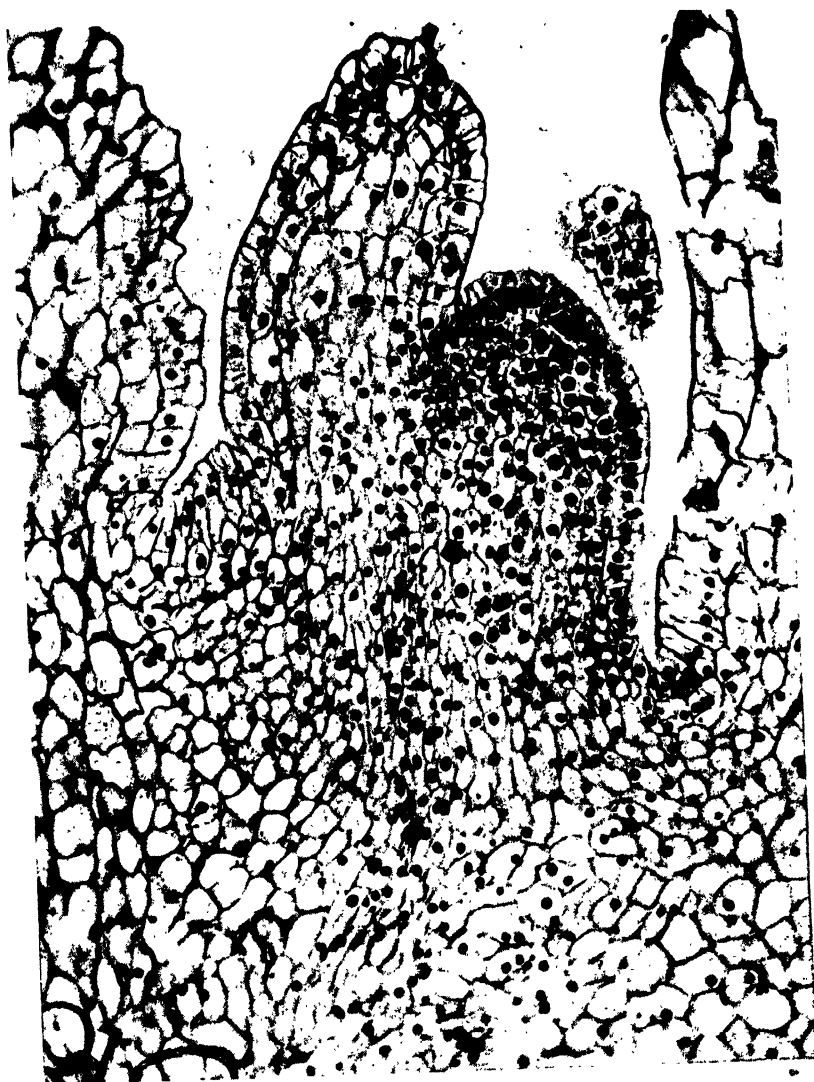


FIG. 13.—*L. harrisii* 14 days after treatment. Axillary hump, through continued proliferation and growth, developed into an axillary bud consisting of growing point and two embryonic leaves. Vascular differentiation beginning near center of figure and extending downward.



FIG. 14.—*L. harrisii* 39 days after treatment; more advanced stage than fig. 13



FIG. 15.—*L. harrisii*, still later stage in bud development than in fig. 14, from stem 39 days after treatment. Apical meristem at center surrounded by embryonic leaves.

### Cytological observations

The regions of response in the three species of *Lilium* investigated show active and fairly rapid divisions of cells. Mitotic figures from these regions are shown in figure 16. The prophases appear to proceed in an orderly and regular manner. The chromosomes then come to lie in the equatorial plate arrangement characteristic of *Lilium*, and a clearly defined spindle is evident. Each chromosome is com-

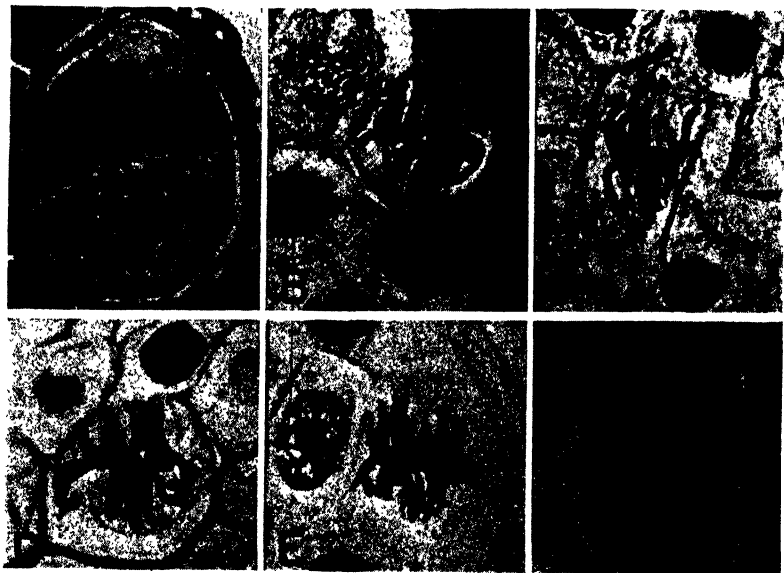


FIG. 16.—Mitotic figures showing regularity of division stages: A, B, D, F, from *L. philippinense formosanum*; C, E, from *L. harrisii*.

posed of two chromatids which separate as the anaphases ensue, and pass through the spindle toward its two poles. The diploid number of chromosomes (24) appears to be present without exception. No evidence indicating the failure of separation of chromatids and no suggestion of fragmentation or other irregularities in behavior have been seen in the cells of either the induced roots or shoots. The observations indicate that the mitotic figures are as distinct as in the cells of root or stem tips taken from plants grown under ordinary garden conditions, and lead to the conclusion that the divisions are perfectly regular in all respects.

### Discussion

Previous reports dealing with the effects of growth promoting substances on higher plants have indicated the inhibition or marked retardation of lateral bud development when the terminal bud is excised and the substance is applied to the cut surface of the stem. In experiments on certain species of *Lilium*, one (*L. harrisii*) has been found in which lateral bud formation may be induced by indoleacetic acid. At the time of treatment the stems showed neither buds nor bud primordia in the leaf axils near the place of application. One to as many as three buds per leaf axil may be induced by the treatment. They develop in the first, second, and/or third leaf axil nearest the surface of application. In the other two species used, *L. philippinense formosanum* and *L. longiflorum*, buds were not induced, but in them adventitious roots were produced from approximately the surface of application to as far down as 2.5 cm. from it. Why such variation in response should occur, and why such marked specificity in the regions affected in the different species should occur, is not understood. The stem anatomy of the three species shows no recognizable structural differences and it is difficult to visualize a different path of transport of the growth substance in the species investigated.

GREENLEAF (4) has recently reported the induction of buds, following similar treatment, in several species and species hybrids of *Nicotiana*. A relatively high percentage of the induced shoots in *Nicotiana* show polyploidy, which is in striking contrast to those produced in *L. harrisii* in which no indications of chromosome doubling or other mitotic irregularities have been detected.

It is possible that the polyploid shoots may arise in *Nicotiana* in the same way that they do in its close relative, the tomato, even if the method which causes callus formation, followed by bud development, in the latter does not induce it in the former. The indoleacetic acid applied to the cut surface of decapitated stems may serve as the excitant for callus formation, and once this is developed, the same conditions or factors which operate to cause chromosome doubling or quadrupling in the tomato may become operative in *Nicotiana*. There is no evidence from the experiments on the bean (10), *Iresine*



(7), tomato (2), or *Lilium* that the indoleacetic acid induces polyploidy.

The histological responses of the cells of *Lilium* to indoleacetic acid are strikingly different in some respects from those in the bean and other plants, which develop large overgrowths or tumors in consequence of the treatment. There is a marked limitation of tissues affected, the cells which respond being limited chiefly either to the fundamental parenchyma in the vicinity of the vascular bundles or to the cells of the outer cortex near the leaf axils. In fact there is a definite specificity of region involved in the two types of responses in *Lilium*. New meristematic regions are generated in response to application of the indoleacetic acid, which develop in two of the species into roots and in the other species into stems.

Cytological observations have been made on the cells of the roots and buds induced by the indoleacetic acid treatment. These have been studied in all possible stages of division in almost all the phases of development of the induced structures. Nothing has been observed which offers support to JONES'S (8, 9) suggestion of the "possibility that missing genes due to corresponding deficiencies in certain parts of both members of a chromosome pair result in a chromosomal unbalance and this brings about unregulated growth," nor was anything seen which suggests a condition of deficiency in any of the chromosomes. Neither were there indications of fragmentation, translocations, or other chromosomal irregularities such as are produced by x-rays, radium, hormones, and other powerful physical and chemical agents. On the contrary there was evidence indicating regularity of division and of chromosome structure and behavior throughout all the stages of mitosis.

### Summary

1. The application of 3 per cent indoleacetic acid in lanolin to the cut surfaces of decapitated stems of three species of *Lilium* produced two sharply differing types of response. *L. philippinense formosanum* and *L. longiflorum* responded by the production of adventitious roots from approximately the surface of application to as far down as 2.5 cm. from it, while *L. harrisii* produced buds in the axils of the upper two or three leaves, with no adventitious roots in most cases.

Decapitated untreated stems produced neither adventitious roots nor buds.

2. A few plants of *L. philippinense formosanum* were allowed to develop flower buds. The pedicels of some of the nearly mature buds were cut off about 1 cm. below the receptacles and the cut surfaces smeared with the lanolin mixture. These responded in essentially the same time interval and in much the same way as did the treated stems of this species.

3. A few of the floral buds were permitted to flower. The lanolin mixture was smeared over the unpollinated stigmas of some of the flowers, while in others the ovary was cut across at about 0.5 mm. below the base of the style and the cut surface was then covered with the mixture. Although the treated ovaries enlarged slightly and remained alive for a longer time than did those which were untreated and unpollinated, none of them continued to develop parthenocarpically.

4. Gross observations extending over a period of 3 months were made on treated and untreated stems. Material for histological studies was collected and fixed at 24 hour intervals up to a total of 39 days.

5. In general the three species responded at essentially the same rate, but much more slowly than has been reported for the bean, tomato, and *Iresine*.

6. A small amount of callus may sometimes develop at or on the surface of application, but more often it is wanting.

7. In *L. philippinense formosanum* and *L. longiflorum* the indoleacetic acid produces little or no observable effect upon the cells of the epidermis and outer cortex. The first detectable changes occur in the cells of the fundamental parenchyma, lateral and centrifugal to the vascular bundles. These cells become meristematic, and by repeated divisions give rise to cells which later differentiate as adventitious roots. As a rule only the outer bundles of the stems are involved.

8. As the cells in these regions continue proliferating, and the meristematic masses increase in volume, they encroach laterally and centrifugally upon the fundamental parenchyma which has not responded visibly to the treatment, crushing and digesting these

cells and those of the outer cortex as growth toward the surface proceeds. Before emerging from the stem, a central core of cells is differentiated and an apical histogen becomes evident in the young roots.

9. Following the general blocking out of the root, xylem elements are differentiated near the base and periphery of the inner core of cells. The first xylem differentiated appears always to be tracheids which connect the vascular systems of root and stem. Phloem, which also forms a continuous system between root and stem, is differentiated simultaneously with the xylem but on different radii. New tissues are added from the derivatives of the apical histogen; the root pushes out through the outer cortex and epidermis, and appears normal in all respects.

10. The treated pedicels, while in general responding similarly to the stems, show one rather marked difference. All, or nearly all, of the vascular bundles are associated with the production of adventitious roots. The roots develop in the same way as in treated stems.

11. In *L. harrisii* the region of visible response to the treatment is limited largely to the cells of the epidermis and outer cortex in the immediate vicinity of the leaf axil. The epidermal cells of the stem immediately above the axil elongate radially, while the outer cortical cells centripetal to, as well as slightly below, them enlarge and in the course of about 5 days begin dividing. This marks the initiation of the region at which buds will later develop. Neither buds nor bud primordia are present in the axils of the upper leaves at the time of treatment.

12. The walls of the original parental cells in the regions of response remain distinct, in comparison with those of their derivatives, for a period as long as 12 days. Cell plates, followed by cell wall formation, seem to follow nuclear division in all cases. Multinucleate cells have not been observed in any of the species studied.

13. The groups of meristematic cells in the leaf axils undergo repeated divisions, accompanied by radial divisions of the cells of the epidermis over them. The subsequent growth of their derivatives results in a hump of cells, which through further development and differentiation becomes a bud. From one to as many as three buds may develop in one axil.

14. Vascular differentiation occurs in the induced buds in essentially the same way as in the buds normally produced near the bases of the stems in this species.

15. When permitted to grow on the plants for a period of 8 to 10 weeks, the induced buds develop into bulbils, from the bases of which adventitious roots arise. The bulbils can be removed from the parent plant and grown separately.

16. Cytological study of mitotic figures in both induced roots and buds indicated regular chromosome form and behavior in divisions. There was no evidence of the failure of separation of chromatids, no fragments were observed, and no other irregularities in behavior were detected.

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# HISTOLOGICAL RESPONSES OF MIRABILIS JALAPA TO INDOLEACETIC ACID<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 492

KARL C. HAMNER

(WITH TWENTY-EGHT FIGURES)

## Introduction

This paper is one of a series dealing with the gross and histological responses of plants to growth substances, or hormones. A number of dicotyledonous and monocotyledonous plants have been selected, the basis of such choice being the extent to which the particular species or variety responds to such treatment, and the desire to secure a wide range of anatomical types or tissue patterns. When evidence and data gathered in this way are more nearly complete, a more critical and comprehensive evaluation of the phenomena of cellular and tissue differentiation can be made, and many metabolic processes and changes can be studied in greater precision and detail (3, 12, 13, 15).

Previous reports have included studies on the comparative responses of the plant to various growth promoting substances (4, 5, 6, 9, 10, 11), to treatment of various aerial organs of the plant, to treatment of the cut surface of the severed stem as compared with treatment of the uninjured stem, and to treatment of stems of various ages. It is clear (1, 2, 6, 7, 8) that no single tissue or tissue system responds either qualitatively or quantitatively the same throughout the range of types investigated. It is also obvious that in any given species or variety some tissues are much more sensitive to stimulation and respond to a much greater degree than do others. The relative state of maturity of a cell or cells making up a tissue has much to do with the character and degree of its response, as have also specific environmental conditions. Despite these ranges of behavior, however, there is a definite pattern for each species or variety.

<sup>1</sup> This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation. Additional cost of publication sustained by the writer.

### Investigation

Most of the material used in this investigation was grown during the late summer and early autumn of 1937. Seeds of four o'clock, *Mirabilis jalapa* Linn., were planted in rich garden soil and grown continuously on an evenly lighted bench in the greenhouse. No attempt was made precisely to control temperature or humidity, although the plants were kept well watered and the walks and benches sprayed with water to prevent excessive temperature or dryness. The plants grew vigorously throughout the time the experiments were conducted.

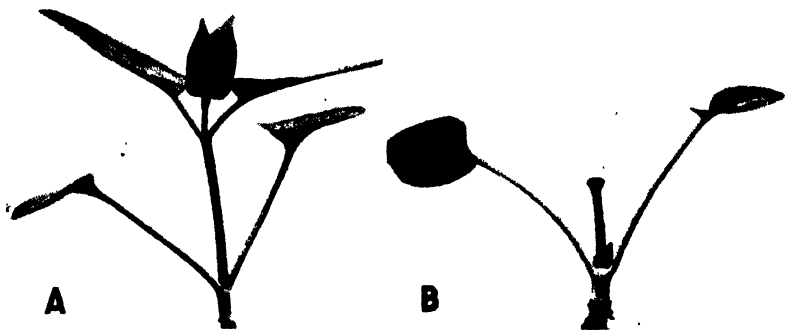


FIG. 1.—A: young plant of *Mirabilis jalapa* at time of decapitation; second internode just starting to elongate. B: similar to A, 8 days after decapitation and treatment with 2% indoleacetic acid in lanolin.

The specific treatments were made when the plants were of two different ages. One group was treated when the first internode of the young plants had elongated considerably and the second internode had just started to elongate (figs. 1A, 3). Another group was treated when the plants were older, the fourth internode having elongated and the fifth just beginning to elongate (figs. 2, 23). In all instances here recorded, treatment consisted of completely severing the stem in the upper portion of the first internode. Stems of the first type showed no outer peripheral secondary thickening, those of the second an appreciable amount (figs. 3, 23). Immediately after such decapitation the cut surface of most of the plants was treated with a small amount of a 2 per cent indoleacetic acid-lanolin paste. A few of the plants were treated with pure lanolin only, and

some were left with the cut surfaces uncovered and exposed. Still other plants were treated with a small ring of the lanolin mixture completely encircling the uninjured stem, which was not decapitated. Since these plants showed the same general types of histological responses, with the exception that the pith parenchyma was much less active than in those decapitated and treated apically, the details are not recorded here. The effects, in so far as they differed from the decapitated plants, were of the same general kind as already indicated for the bean (6, 8) and *Iresine* (7), in which the same types of technique were employed.

Material for histological investigation was collected from each group of plants at various intervals after decapitation and treatment. Navashin's solution was used throughout as a fixative. The butyl-alcohol paraffin method of imbedding was used, and the material sectioned at 10  $\mu$ .

### Gross responses to treatment

The gross responses of the treated stems were similar, whether the fourth internode or only the first was elongated at the time of treatment. Within twenty-four hours the tissues near the treated surface showed a distinct yellowing; at forty-eight hours the tip for a distance of 1 to 1.5 mm. from the treated surface had increased in diameter more than the remainder of the stem. By the end of six days enlargement had increased appreciably and root primordia were visible in vertical rows beneath the surface. At nine days still greater swelling had taken place and the tumor was light yellow or almost white, its surfaces more or less tuberculated, and the young roots were protruding not only from its lateral surfaces but often also from the upper surface where the lanolin mixture had been applied. After the fifteenth day the tumor may continue to enlarge slightly and the young roots protrude somewhat farther, the degree of such emergence and continued development depending directly upon the atmospheric humidity. If the air is nearly saturated, the roots become long and threadlike; if it is dry they protrude scarcely beyond the surfaces of the tumor and many of them do not emerge at all. The tumor may continue growth for at least one hundred days, but during this time there is no great proliferation of tissues



FIG. 2.—Young plant with fourth internode elongated. Such plants were severed near top of first internode and treated with lanolin mixture. They differ mainly from those shown in fig. 1 in that secondary thickening in treated region is somewhat advanced and all primary tissues are more nearly mature (see figs. 23, 24).



to produce a large apical tumor above the treated surface, as in the bean. Although the lateral shoots in the axils of the cotyledons are inhibited in development for several days, they generally grow rapidly after the sixth day following application of the lanolin mixture. After growth has been initiated they develop much as do shoots in untreated plants, and overlap the decapitated central axis with its terminal tumor which, as stated, may persist for many weeks but does not usually become more than 1 cm. in diameter. In some plants, however, the lateral shoots may be almost completely suppressed. In such instances the cotyledons become thick and fleshy, and persist for at least four or five months. The hypocotyl increases markedly in diameter, much more rapidly than in decapitated untreated plants, and also more than in check plants which were not decapitated but allowed to grow without any type of treatment other than watering. The tumors on these plants generally become relatively large, more or less globular, green, with scurfy surfaces. Two of them attained a diameter of more than 2 cm. by the end of five months, and were apparently still growing when harvested. None of the plants was given more than the single initial treatment with the lanolin mixture. The tumors seldom extend for any considerable distances down the stem. They are more or less flat topped, sides bulging slightly, and these terminate below rather abruptly. Below this sharp taper the stem as a whole shows very little change from the untreated stem of a non-decapitated plant, except that when lateral shoots develop from the cotyledonary node, the first internode (except for the tumor) does not continue to undergo secondary thickening so rapidly nor to so great an extent as that of the undecapitated plant.

The first internodes of plants decapitated when the second internode is just beginning to elongate and treated with pure lanolin never produced tumors. None of them increased in diameter following decapitation nor showed any additional secondary thickening. Some of them developed a very weak phellogen-like layer just below the treated surface, remained green for about two weeks, and then the whole first internode became excised following formation of an abscission layer just above the cotyledonary node. All of the decapitated and untreated first internodes became abscised in this way

within two or at most three weeks following decapitation. There was practically no subsequent increase in cell number or cell size, although the internodes remained green and turgid for seven to ten days. After that time they became yellowed and dropped from the plants either while still fairly turgid or after becoming very much shriveled (fig. 6A, B). In every instance shoots developed in the axils of the cotyledons and after three or four weeks the cotyledons became yellow and gradually withered or were abscised.

### Histological details

#### ANATOMY OF UNTREATED STEMS

*Mirabilis jalapa* is similar in stem anatomy and in type of secondary thickening to forms in the Chenopodiaceae and Amaranthaceae which have been described by WILSON (16). At the time the first internode has elongated considerably and the second internode has elongated but slightly (fig. 1A), a cross section of the first internode just below the second node shows very little secondary thickening (fig. 3). The stele is bounded peripherally by an endodermis or starch sheath. The pericycle consists of a layer or two of cells just inside the endodermis. Roughly there appear to be two sets of vascular bundles, each set arranged in the form of an ellipse. The inner ring consists of from eight to ten larger vascular bundles surrounded by or scattered through the pith. The outer ring consists of many smaller bundles some of which lie just inside the pericycle and others several cell layers inside the pericycle. Each of these bundles may possess a fascicular cambium from which both secondary xylem and phloem may be derived, although usually the amount is not great. Between each of these smaller bundles are a number of parenchymatous cells, and from these cells an interfascicular cambium may be developed, a cambium becoming continuous laterally either with the fascicular cambium of some of the smaller vascular bundles, with a cambium derived from the pericycle over the phloem of such a bundle, or with both such cambiums (fig. 4).

There is very little cambial activity in the vascular bundles in which the fascicular cambium is not continuous with the interfascicular cambium, but that portion of the cambium which is

present in the small outer bundles and which is continuous with the interfascicular cambium may be much more active and considerable secondary xylem and secondary phloem may be differentiated from it (fig. 5). The interfascicular cambium itself is highly meristematic, and from its derivatives there differentiate the conjunctive tissue masses of isolated tracheae and tracheids, isolated phloem elements, and additional vascular bundles with an active cambium remaining between the xylem and phloem portions (fig. 5). At about the time the fourth internode has elongated, a cross section of the first internode shows very little secondary thickening in any of the bundles except for the outermost ones (figs. 23, 24), which have an appreciable amount of secondary xylem and phloem, and between them there is a considerable amount of conjunctive tissue.

Shortly after the stage shown in figures 23 and 24, the activity of the fascicular cambium may cease in some of the vascular bundles and a cambium develops in the pericycle over the vascular bundles and becomes continuous with the interfascicular cambium (fig. 4). Division continues in the entire ring of cambium around the stem (figs. 4, 5). Under ordinary circumstances the cambium in the larger bundles of the ellipse nearer the center of the stem is never highly meristematic; even stems six to eight months of age, as shown in figure 5, have very little of either secondary xylem or phloem. In stems treated with lanolin mixture there is much less response by the cambium in these bundles than in the peripheral cambiums.

If the stem is severed across the first internode at the time the second internode is just beginning to elongate and the cut surface is not treated or is treated with lanolin only, there is practically no additional meristematic activity in the remaining cells of the first internode (fig. 6). They may remain alive and the internode change little from the time of decapitation (fig. 6A) until it is abscised, or many of its cells may die and the whole stem become shriveled (fig. 6B).

#### CHANGES IN PLANTS TREATED WHEN ONLY FIRST INTERNODE HAS ELONGATED

Following decapitation and treatment of the cut surface with the lanolin paste, little histological response was shown during the first

two and one-half days. The portion of the first internode below the treated surface appears in cross section (fig. 7*A*) very similar to the plant at the time of treatment (fig. 3). Some of the cells of the pericycle and interfascicular parenchyma show rather large nuclei and dense cytoplasm. Four days after treatment (figs. 7*B*, 8*A*, *B*) marked changes have taken place a short distance below the treated surface. Cells of the cortex, pericycle, and interfascicular parenchyma in some portions of the stem are actively dividing (figs. 7*A*, 8*B*); in other portions little change is apparent (fig. 8*A*). Some divisions have taken place in cells of the endodermis, and in cells of the pith nearer the center of the stem.

Subsequent developments are indicated in figures 7 to 21. Studies of a number of plants were made at regular intervals following treatments. Only those examples which show significant progressive changes are shown. In general there was decided uniformity in response but not every plant responded at the same rate. Certain of the plants, although only the first internode had elongated at the time of treatment, had much thicker stems than had others, and these larger stemmed plants responded more rapidly than those with a stem of smaller diameter. A careful study of the type of response, however, showed no essential difference in the pattern. Both types of plants have been used for the illustrative material.

In every case the portion of the stem from 1 to 3 mm. below the treated surface responded most rapidly, resulting in a spindle-shaped tumor in about seven days. From this region most of the adventitious roots appeared. Subsequently tissues of the pith adjacent to the treated surface began active division, producing masses and small tubercles of tissue extending above the plane of the original cut surface. By fourteen days following treatment the tumor was roughly hemispherical. In practically every case, the only response to the treatment exhibited by the portion of the stem more than 6 mm. below the treated surface was growth and development of a type such as might occur if the plant had not been decapitated and treated. Except for the consideration of the responses of the pith, the discussion of the responses of the separate tissues which follows will be confined to the response in that portion of the internode from 1 to 3 mm. below the treated surface. If a response was

obtained above or below this region in a given tissue, it was usually of lesser degree.

In none of these experiments has the epidermis shown marked response to the treatment. The cells may enlarge somewhat and few radial divisions may occur, but no tangential divisions have been observed. As the result of the growth of tissues underneath it, the epidermis may be stretched and ruptured and a few weeks after treatment portions of it may be dead.

The cortex shows definite response but, except for the endodermis, never becomes highly meristematic. Many divisions may occur throughout the cortex, but even in the largest tumors it does not constitute a conspicuous portion of them. As the result of active cell division and growth in the endodermis and stele, portions of the cortex may be ruptured and many of its cells die. In no case was there observed a cork cambium such as appears in the cortex of old, untreated, and undecapitated plants (fig. 5).

The cells of the endodermis divide and produce many parenchymatous cells as a result of treatment. By far the greatest number of cell divisions occur in that portion of the endodermis peripheral to a developing adventitious root, and these are largely radial, the endodermis forming a layer of meristematic tissue capping the tips of the young roots (figs. 9, 10, 11, 13). Activity in the endodermis never becomes so great as that in the tissue just inside it, nor does it form a wide band of meristematic tissue. Derivatives from it have not been observed to differentiate as strands of xylem and phloem as in red kidney bean (8).

Perhaps the most sensitive tissues are the pericycle and the interfascicular parenchyma. Divisions in the cells of the pericycle were apparent within four days after treatment (figs. 7*B*, 8). Many of the adventitious roots formed in response to the treatment were differentiated almost entirely from the derivatives of this tissue (figs. 8, 11). Other adventitious roots were differentiated in a mass of meristematic tissue containing derivatives of both the pericycle and interfascicular parenchyma. This reaction of the pericycle is in contrast to its limited response in red kidney bean (6, 8) and tomato (3).

The interfascicular parenchyma lying just within the pericycle

and between the outer ring of vascular bundles is about equally as sensitive as the pericycle. It is in this tissue that the interfascicular cambium of undecapitated and untreated plants develops. Its cells have become actively meristematic within four days after treatment, and form, together with the meristematic band produced by the pericycle, a mass of meristematic tissue in which many adventitious roots are differentiated and which grow outward through the cortex and epidermis. Other derivatives of the proliferation of the interfascicular parenchyma may remain parenchymatous, or they may form provascular strands which differentiate as vascular bundles connecting the stele of the developing roots to the vascular tissues of the stem, or they may form vascular strands connecting those present in the stem at the time of treatment.

The fascicular cambiums of the various bundles of the stem are not equally responsive. There is little if any response noticeable on the part of the cambium of any of them except that present in the small outer bundles which abut the pericycle. In these the cambium, which corresponds to that portion of the fascicular cambium of undecapitated plants which becomes continuous with the interfascicular cambium, is more sensitive. Following treatment it proliferates, producing a broad band of parenchymatous cells between the xylem and phloem. The xylem and phloem of these small bundles may become crushed as the result of growth and division in the cells around them (fig. 11). In other instances the fascicular cambium shows very little activity (figs. 13, 16), and occasionally additional phloem and xylem elements are differentiated, although frequently no more than in untreated stems.

The cells of the xylem and phloem of the bundles respond little or not at all to the treatment. Some of the parenchymatous cells may enlarge somewhat, but such divisions as occur are very few. This behavior, too, is in marked contrast to the bean and tomato.

Cells of the pith respond more slowly than do cells of the pericycle, interfascicular parenchyma, and the cambium of the small outer bundles; but after activity has been initiated the cells proliferate rapidly and activity continues over a period of many weeks (figs. 12, 18, 21). A few divisions may be observed the fourth day after treatment but the greatest activity begins about the seventh

day (fig. 13). At this time many root primordia have been differentiated in the peripheral portions of the stele. Shortly after root primordia are discernible in derivatives of the pericycle and interfascicular parenchyma, provascular strands may be formed from the derivatives of the pith and these strands connect the stele of the developing root with both the inner and outer vascular bundles of the stem (figs. 13, 20). At about this time divisions of cells throughout the pith may take place, but the greatest activity occurs in those cells surrounding the vascular bundles and in the provascular strands which have differentiated between and interconnecting various vascular bundles of the stem. In this way a complex anastomosis of provascular strands, stem bundles, and adventitious roots is established (figs. 17, 20). Some of these derived vascular bundles run longitudinally in the stem parallel to the original bundles, and are closely anastomosed with them (fig. 15). In some stems adventitious roots are differentiated from the derivatives of actively dividing pith cells adjacent to the outer vascular bundles (fig. 18) or the inner vascular bundles (figs. 22, 27, 28). Such root primordia may differentiate centripetally, laterally, or centrifugally to the bundle and the derived root may grow in almost any direction through the pith, through the surrounding tissues, or outwardly (fig. 18).

The cells of the pith adjacent to the vascular bundles continue meristematic activity for many weeks, and the cells so derived may appear as lumps and overgrowths of intermingled parenchymatous and vascular tissue on the upper surface of the treated portion (fig. 19). Except for the differentiation of roots from derivatives of the pith, this tissue responds much as did the pith of red kidney bean, and constitutes the main body of the very large old tumors previously mentioned.

#### RESPONSES OF PLANTS TREATED WHEN FOURTH INTERNODE HAS ELONGATED

In general, those plants which were decapitated and treated at the time the fourth internode had elongated did not produce so vigorous a response as did the younger plants. At the time of treatment the first internode of these plants (figs. 23, 24) possessed a considerable amount of secondary tissue, whereas the smaller plants had

very little (fig. 3). This secondary tissue consisted almost entirely of an outer band of conjunctive tissue. There was some secondary xylem and secondary phloem in the smaller outer bundles whose cambium was continuous with the interfascicular cambium (fig. 24). All other bundles of the stem had very little secondary tissue.

The most sensitive tissues of these stems were the pericycle, the pericyclic cambium, and the interfascicular cambium. As the result of rapid divisions in these three tissues, within six days after treatment there were masses of meristematic tissues in areas lying between the endodermis and the band of conjunctive tissue. In these masses root primordia were differentiated (fig. 25). In some instances the cambium of one of the smaller outer bundles responded by active cell division, in which case its derivatives took part in the differentiation of developing roots. In all examples in which roots were developed in the tissues just described, differentiation of provascular strands from the stele of the root proceeded inward only as far as the band of conjunctive tissue (fig. 26). No vascular connections were observed between these roots and the bundles centripetal to the conjunctive tissue.

The tissues of the epidermis, cortex, and endodermis responded in much the same manner as described for the younger plants. In contrast to the response of the latter, there was no observable response of the interfascicular parenchyma which might still persist central to the conjunctive tissue. There was no apparent response of the tissues of the vascular bundles with the exception of the cambium of a very few of the small outer ones, as has previously been described.

The pith responded to treatment in a manner similar to that of corresponding areas in the younger plants. The cells surrounding the vascular bundles were the most sensitive and as a result of active divisions of them and in localized areas of other portions of the pith, strands of meristematic tissue extending from bundle to bundle and longitudinally in the stem were formed and these later differentiated as vascular bundles. In certain regions derivatives of the pith cells adjacent to the vascular bundle became organized as root primordia (figs. 27, 28), which upon development formed roots extending in various directions through the pith.



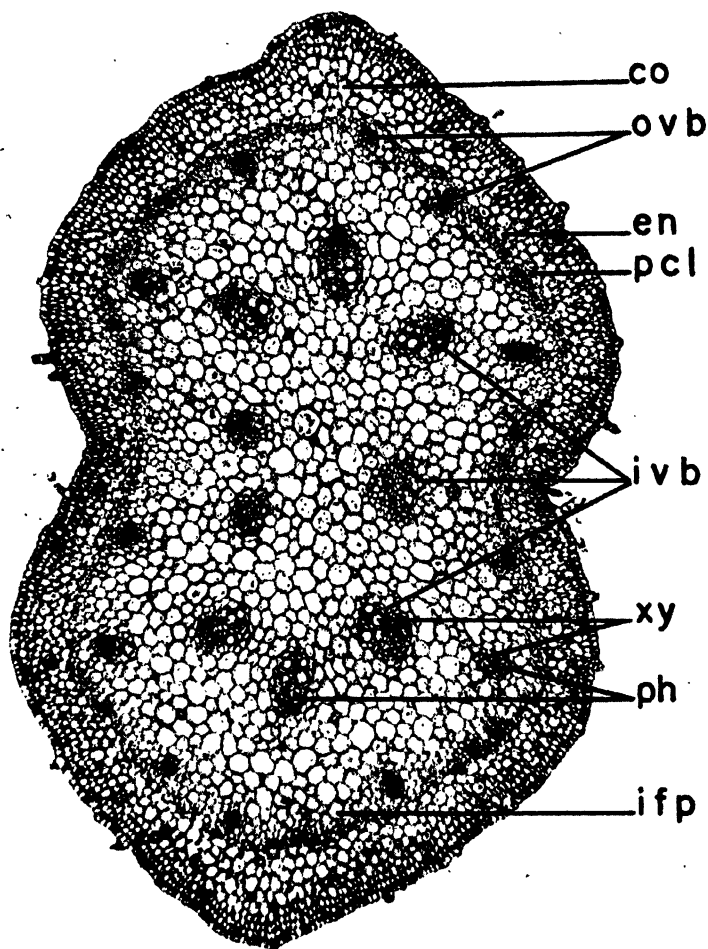


FIG. 3.—Cross section of first internode of plant as shown in fig. 1A. *co*, cortex; *en*, endodermis; *pcl*, pericycle; *xy*, xylem; *ph*, phloem; *ivb*, inner vascular bundles; *pi*, pith; *ovb*, outer vascular bundles; *ifp*, interfascicular parenchyma.

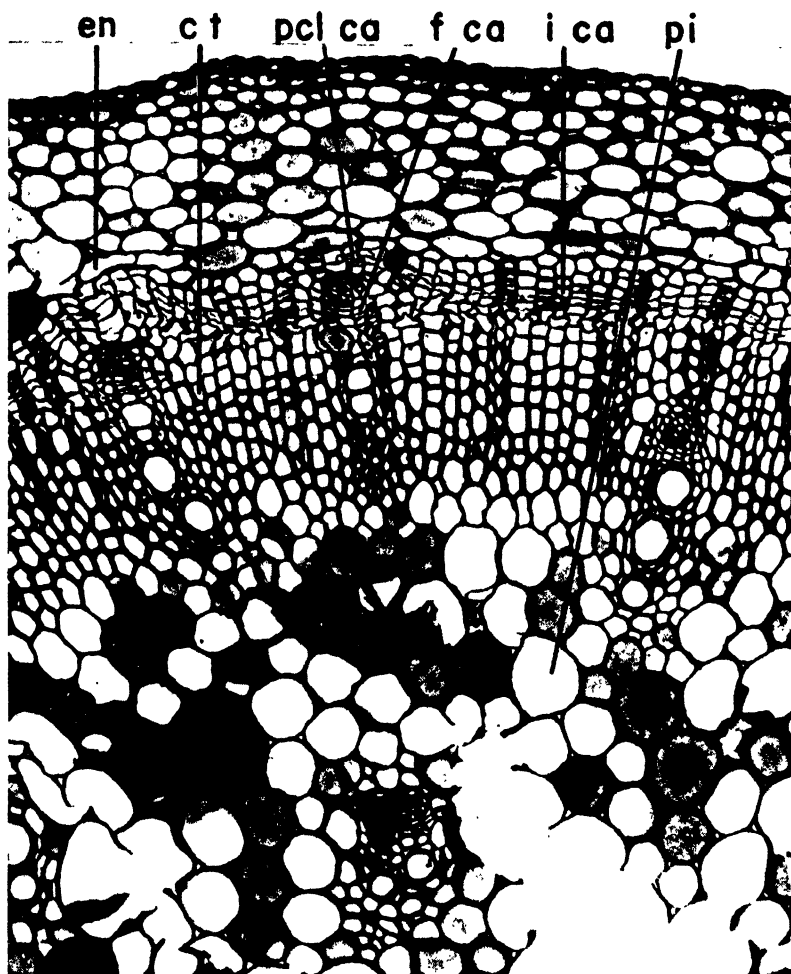


FIG. 4.—Cross section of first internode of untreated plant sixth internode of which is elongating. Considerable conjunctive tissue has been derived from interfascicular and pericyclic cambiums, and secondary xylem and phloem from fascicular cambium of some of outer bundles. A cambium, continuous with the interfascicular cambium, has been derived from the pericycle over the two bundles at upper left hand. *en*, endodermis; *ct*, conjunctive tissue; *pcl ca*, pericyclic cambium; *f ca*, fascicular cambium; *i ca*, interfascicular cambium; *pi*, pith. Inner bundle at lower center showed but few secondary elements derived from cambium.



FIG. 5.—Cross section of basal region of stem several months old. *ck ca*, cork cambium; *ct*, conjunctive tissue; *co*, cortex; *pi*, pith; *vb*, vascular bundle. Most of secondary tissue of stele derived from pericyclic and interfascicular cambiums.

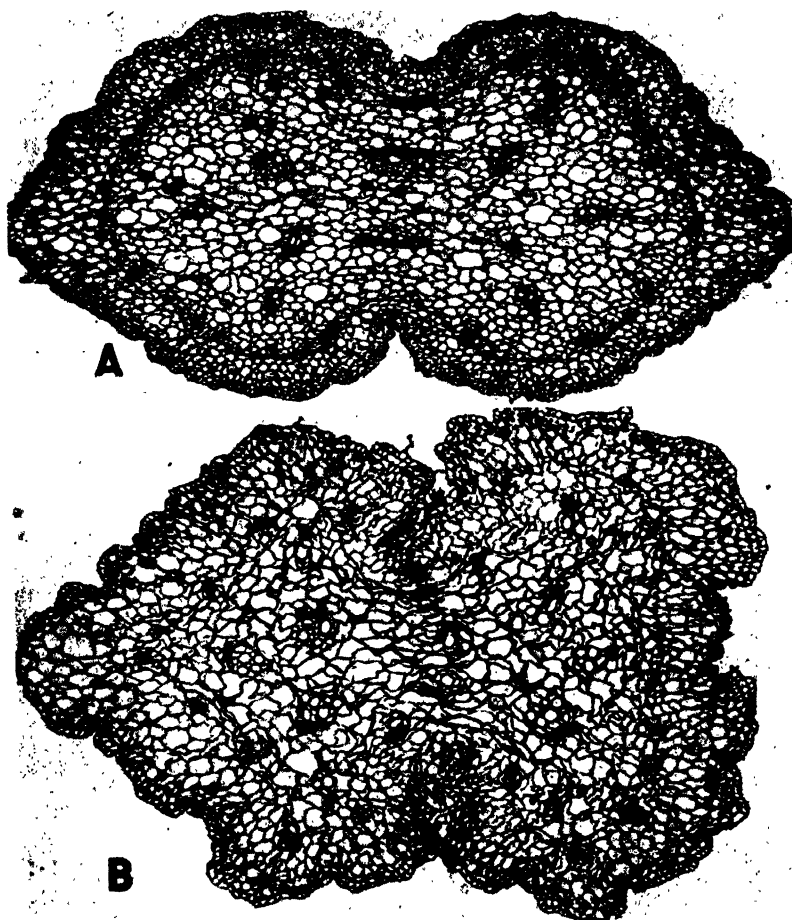


FIG. 6.—Cross sections of untreated first internodes 13 days after decapitation. *A*: most cells of tissues have remained alive but there has been little meristematic activity and no secondary thickening. *B*: same type of stem; many cells dead and collapsed. *B* is slightly more highly magnified than *A*. Cf. fig. 18 which is about the same age but had been treated with lanolin mixture, and fig. 23 which is also about the same age but was not decapitated or otherwise treated.

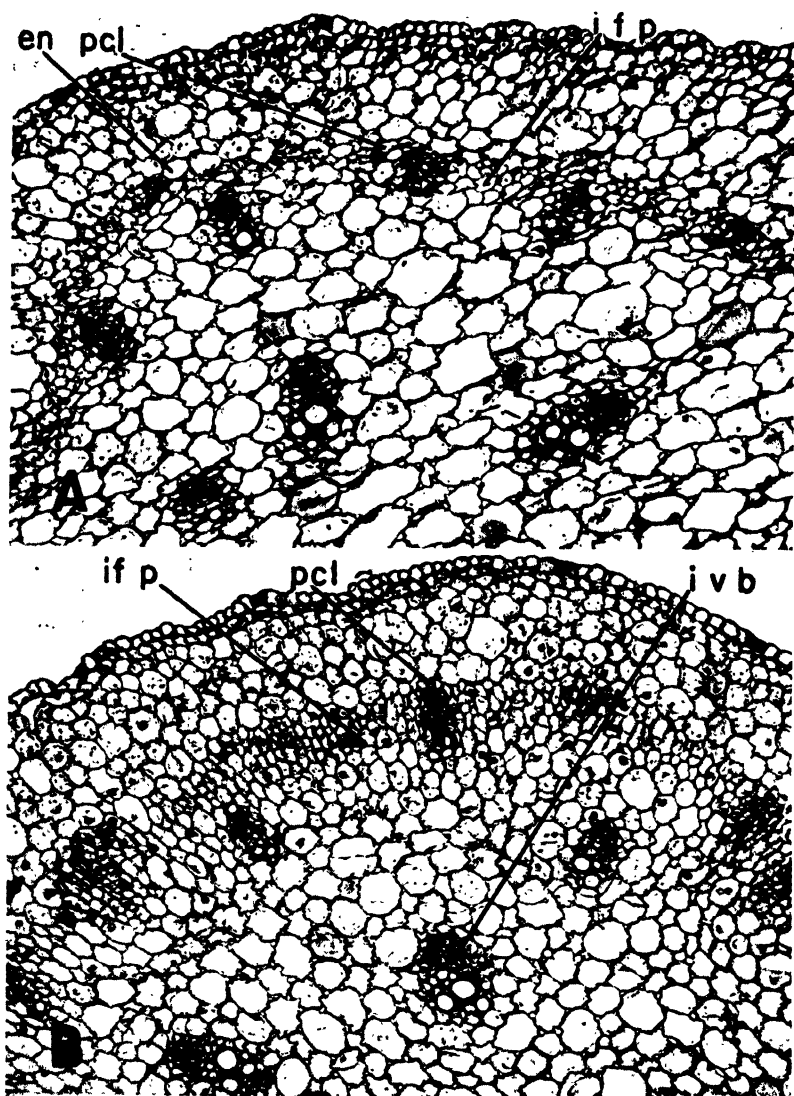


FIG. 7.—*A*: 60 hours after treatment, section about 1 mm. below treated surface; some activity of fascicular cambium and of phloem parenchyma. *B*: 96 hours after treatment, section about 1 mm. below treated surface. Cells of phloem, cambium, pericycle, and interfascicular parenchyma actively dividing. Some divisions in endodermis, cortex, and pith. *en*, endodermis; *pcl*, pericycle; *ifp*, interfascicular parenchyma; *ivb*, inner vascular bundle.

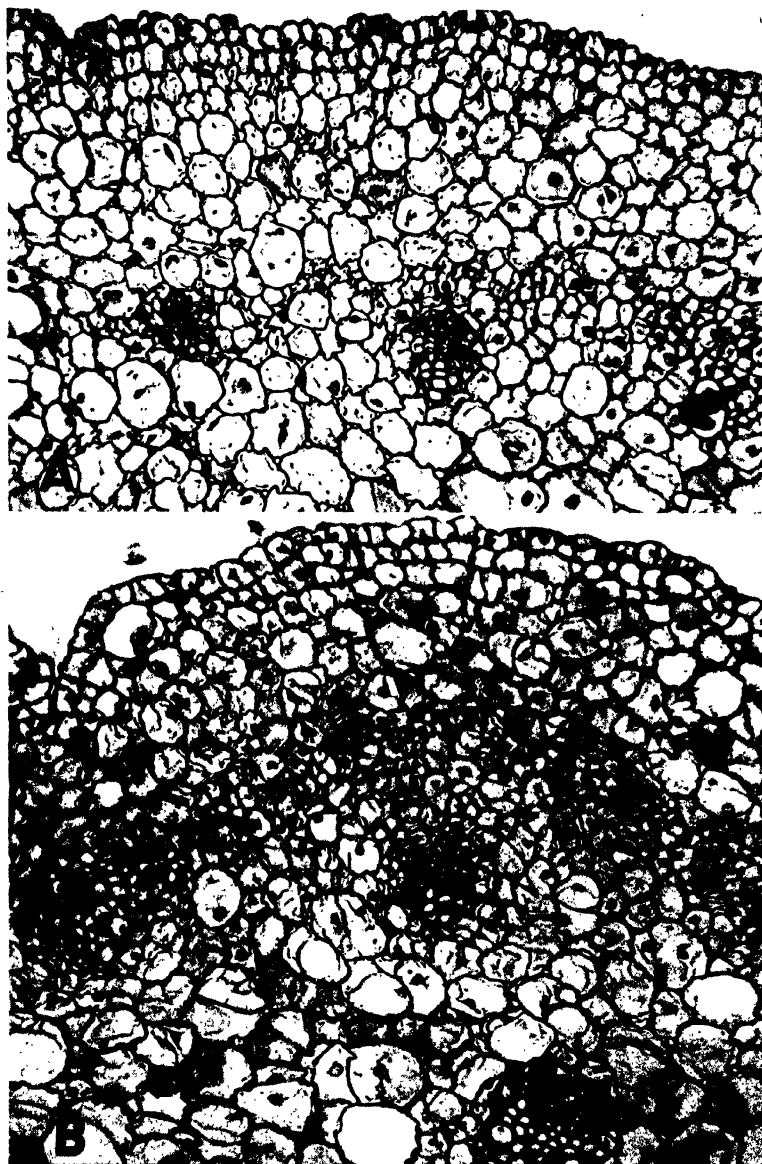


FIG. 8.—Ninety-six hours after treatment, 1 mm. below treated surface. *A*: sector of stem showing relatively little activity. *B*: of same section showing much greater activity; interfascicular parenchyma and pericycle especially active.

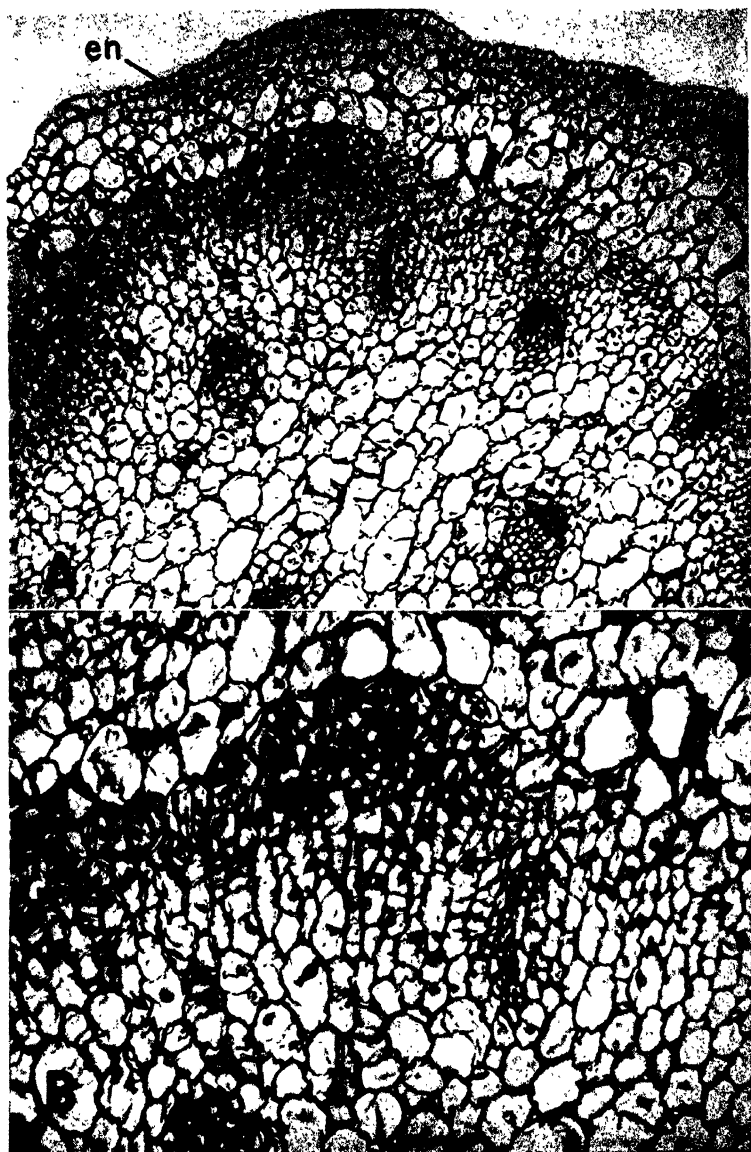


FIG. 9.—Five days after treatment, showing beginning of root primordium as derived mainly from interfascicular parenchyma and pericycle. Endodermis actively dividing; its derivatives form a cap over the primordium. *B*: enlarged view of portion of *A*.

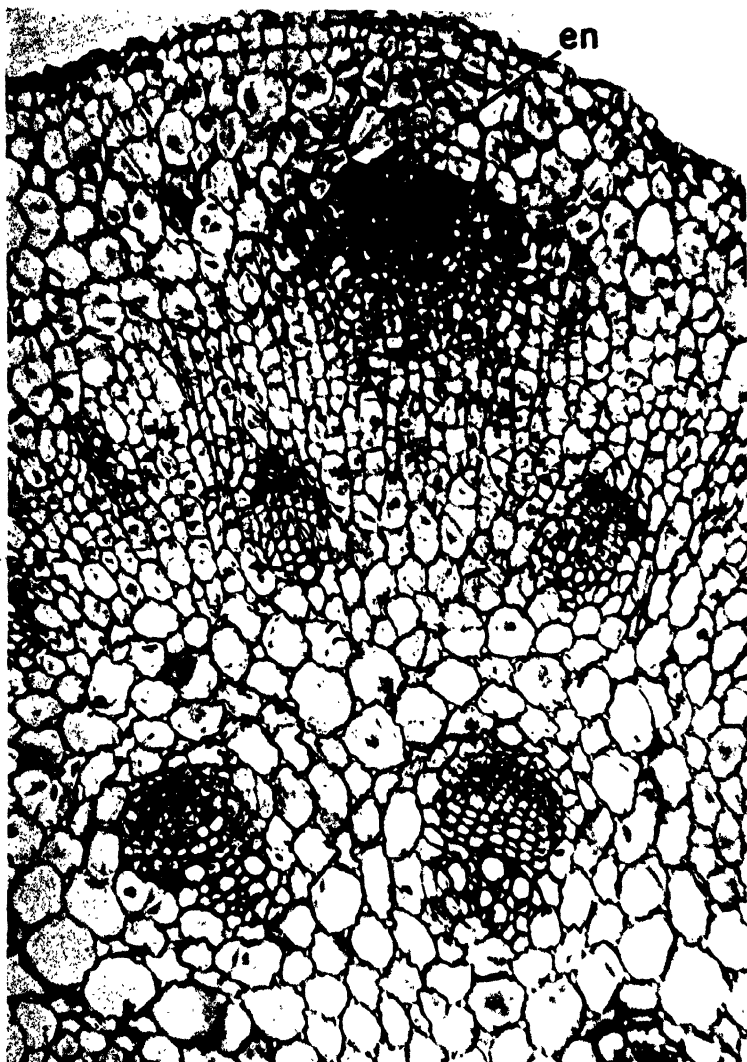


FIG. 10.—Six days after treatment, 1.5 mm. below treated surface. Root primordium from derivatives of pericycle and interfascicular parenchyma. Little activity of pith or cells of vascular bundles. *en*, endodermis.



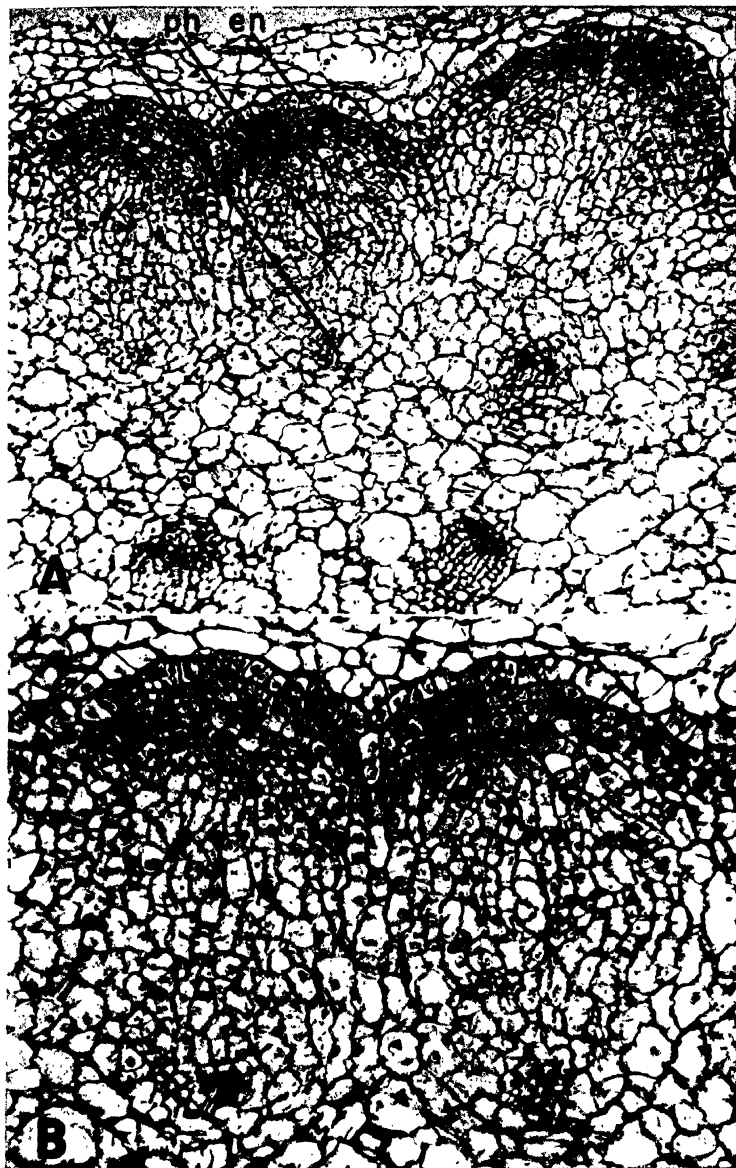


FIG. 11.—Six days after treatment, section about 1.5 mm. below treated surface. *A*: sector showing three root primordia directly over small outer vascular bundles and differentiated mainly from derivatives of pericycle. Phloem and xylem of two outer bundles at left separated by active cells; such activity not manifest in outer bundle at right nor in inner bundles. *en*, endodermis; *xy*, xylem; *ph*, phloem. *B*: section of *A* enlarged.

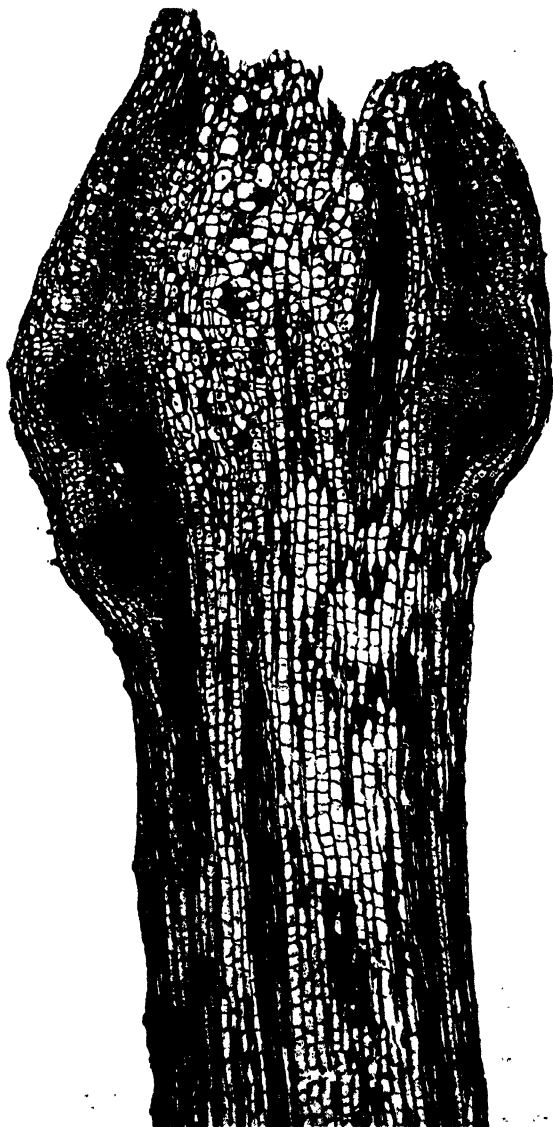


FIG. 12.—Median longitudinal section, 6 days after treatment. Activity greatest 1.5 to 3 mm. below treated surface. Three root primordia shown.

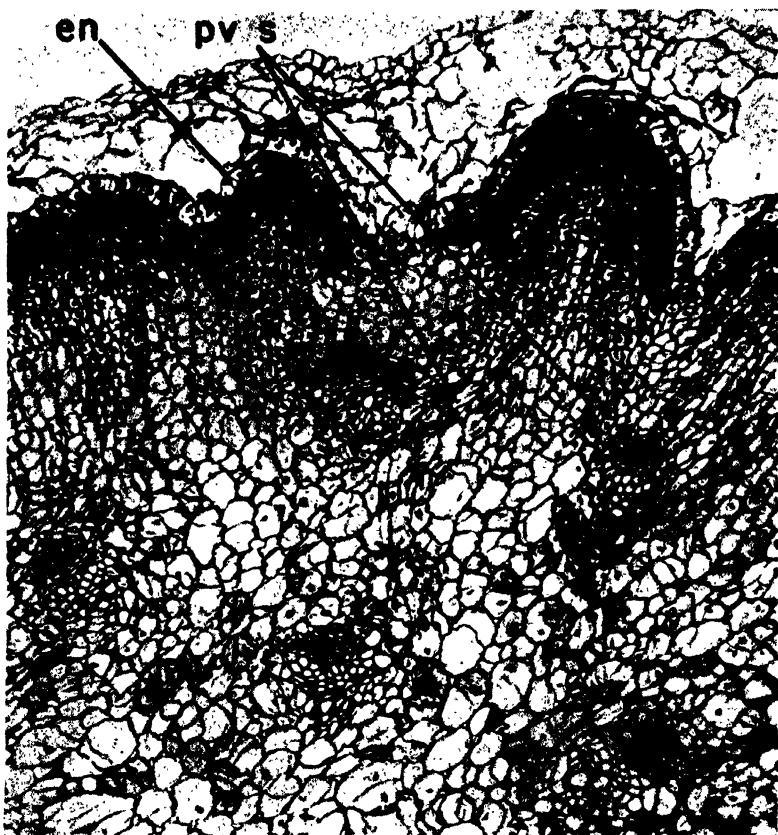


FIG. 13.—Seven days after treatment. In root at upper right, provascular strands differentiating to two outer vascular bundles adjacent to interfascicular parenchyma from which root was mainly derived, and also inward to inner bundle at lower center. Parenchymatous cells around the various vascular bundles actively dividing. *pvs*, provascular strands; *en*, endodermis.

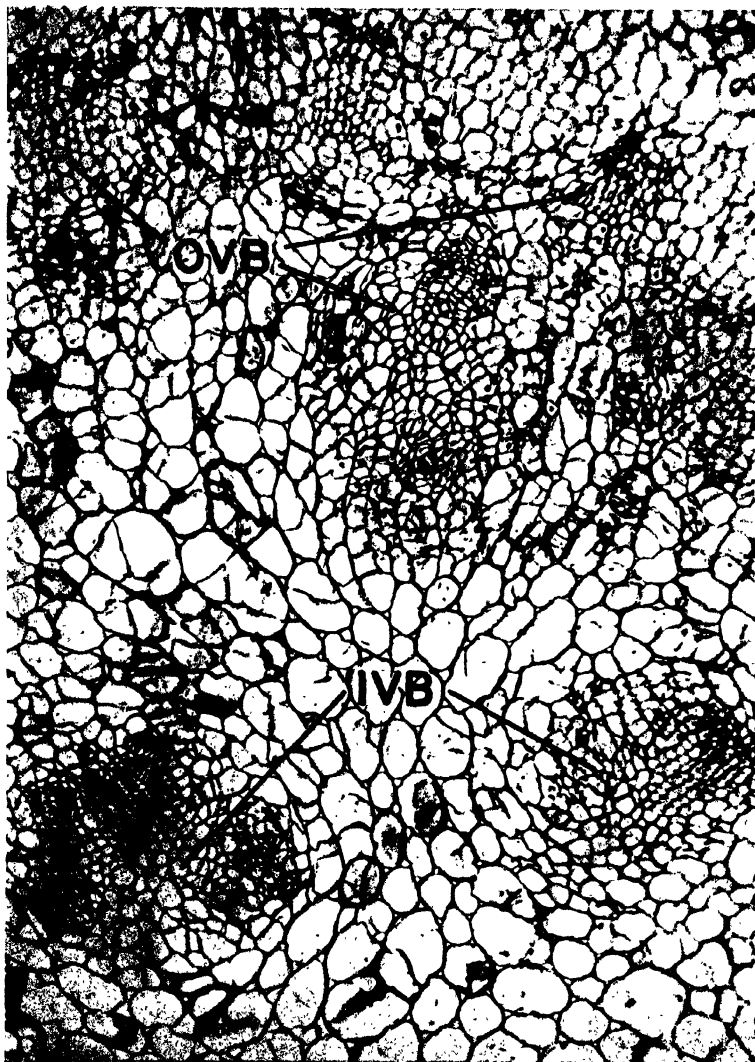


FIG. 14.—Nine days after treatment. Enlarged view of small portion of stem showing two inner vascular bundles and several outer ones. Parenchyma surrounding vascular bundles has actively divided, resulting in strands or islands of meristematic tissue; some derivatives have matured as scattered phloem and xylem elements, and strands adjacent to xylem of original bundles. *ivb*, inner vascular bundles; *ovb*, outer vascular bundles.

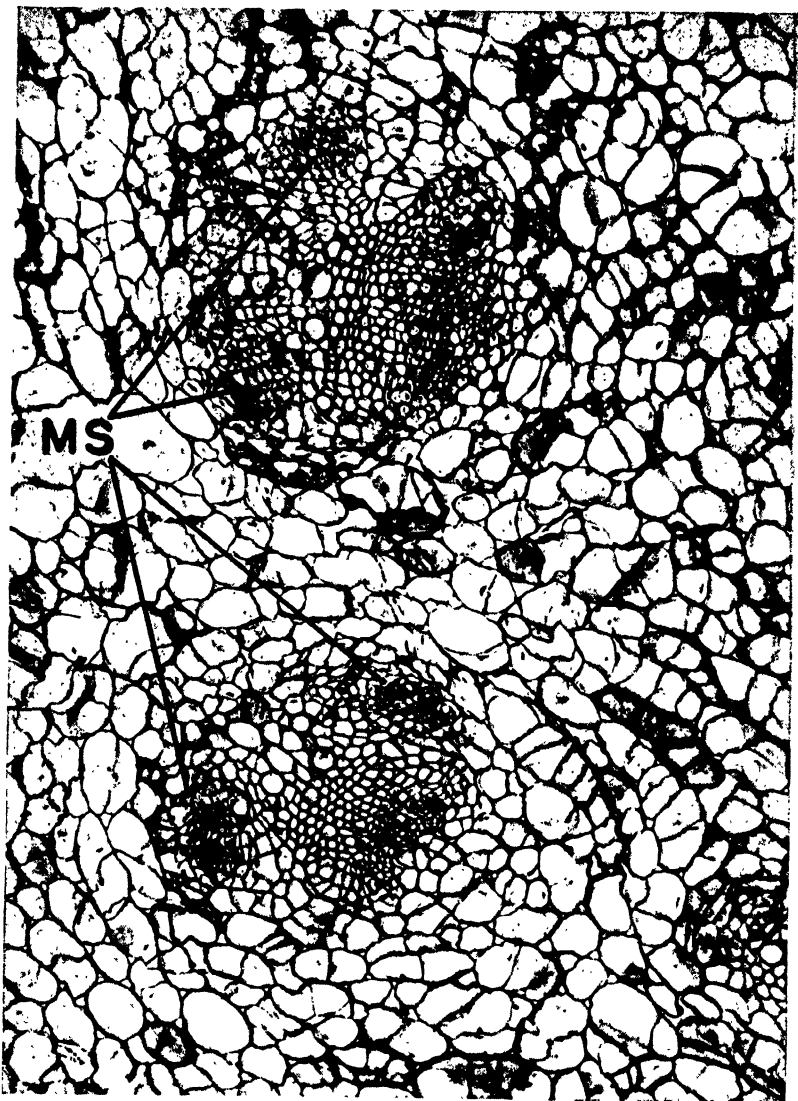


FIG. 15.—Enlarged view of meristematic pith and two of inner vascular bundles similar to those in fig. 14; vascular strands differentiated from derivatives of pith. Cambium of original inner vascular bundles only slightly active.



FIG. 16.—Eleven days after treatment. Root on left has developed directly centrifugal to one of small outer vascular bundles and is comparable with primordium shown at right in fig. 11A. Differentiation of strands of tracheids around phloem of a bundle to the xylem of the same bundle. Root on right has developed from interfascicular parenchyma between two of the outer vascular bundles and is comparable with primordium shown at right in fig. 13. Vascular connection established to each of the two adjacent bundles.



FIG. 17.—Eleven days after treatment. Meristematic strands from which tracheids and other xylem and phloem elements are being differentiated extend in various directions, establishing vascular continuity from bundle to bundle and longitudinally up and down the stem. *Cf.* fig. 15.



FIG. 18.—Cross section, 13 days after treatment. In addition to roots developing outwardly, others have been initiated from derivatives of parenchyma adjacent to xylem portions of outer vascular bundles, and have penetrated inward toward center of stem.



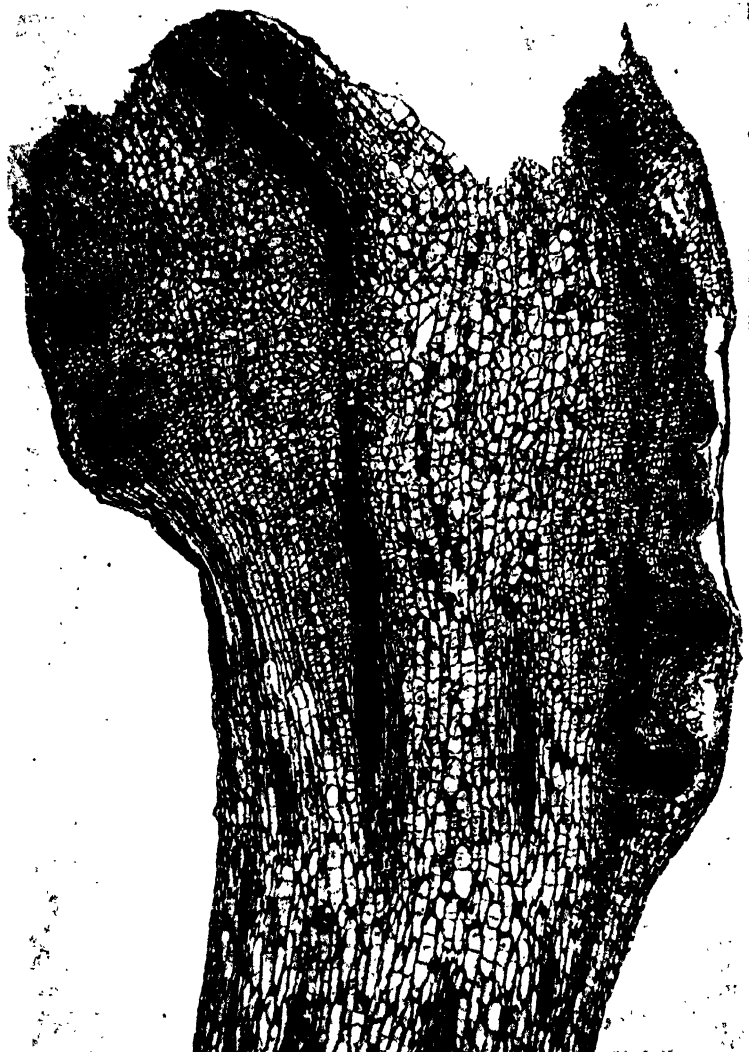


FIG. 19.—Median longitudinal section of stem 17 days after treatment. In addition to numerous young roots in longitudinal series down the stem, pith surrounding the vascular bundles is highly meristematic.



FIG. 20.—Twenty-two days after treatment. Cross section of portion including base of root and several vascular bundles. Vascular connections have been differentiated from the root to the vascular bundle at center and to a small bundle adjacent to it, the adjacent bundle having been differentiated from derivatives of pith near xylem of stem bundle, as shown in figs. 15 and 21.

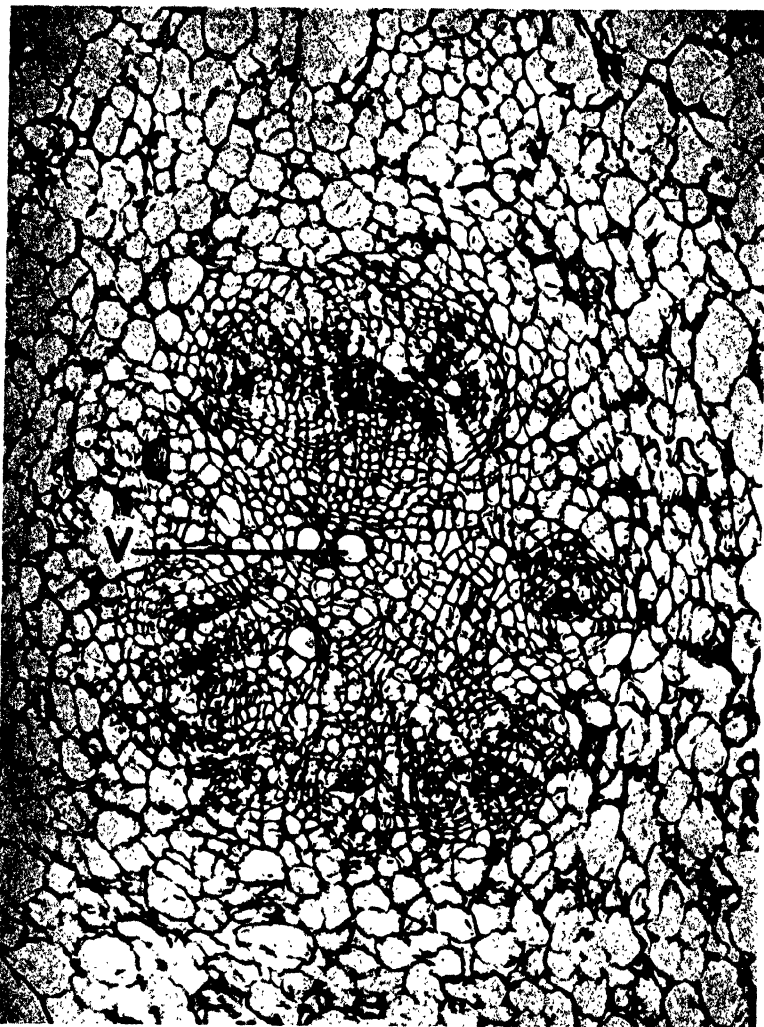


FIG. 21.—Twenty-eight days after treatment. Cross section of portion including one of inner vascular bundles. Parenchymatous cells surrounding bundle are highly active, especially those adjacent to vessels of primary xylem. From derivatives of these cells strands of meristematic cells and vascular elements have been derived. Parenchymatous cells in bundle are slightly active. *v*, vessels.



FIG. 22.—Twenty-eight days after treatment. Parts of two roots shown, each of which has developed from derivatives of actively dividing pith parenchyma located on either side of inner vascular bundle shown at center. *Cf.* fig. 18.

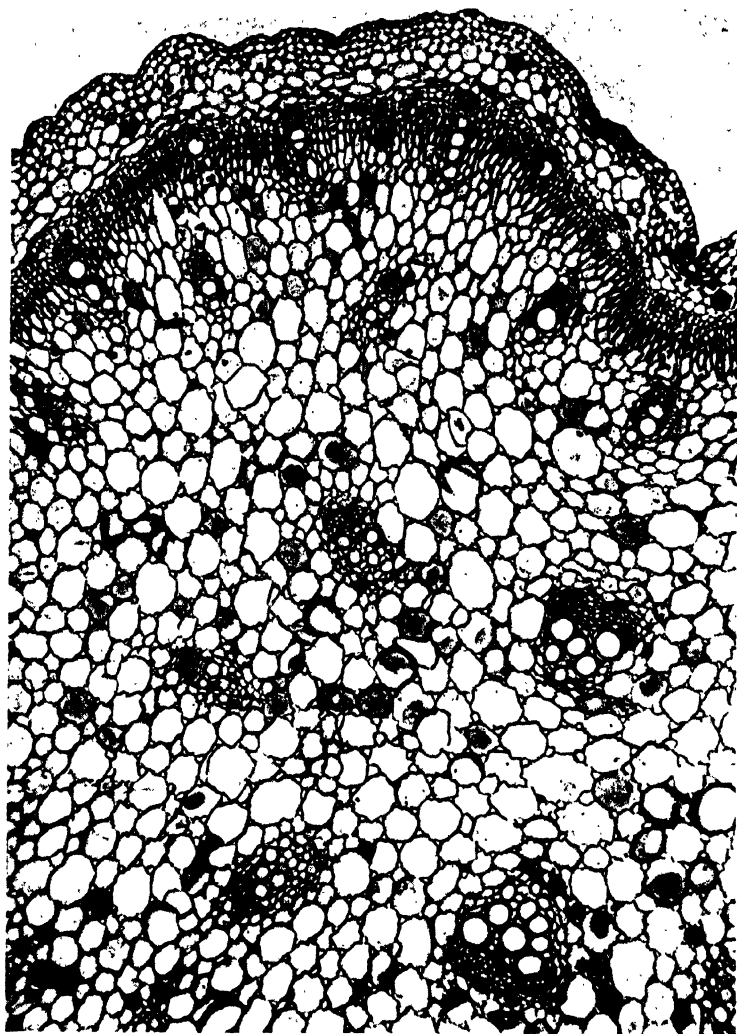


FIG. 23.—Transsection of first internode of untreated plant, fourth internode of which has elongated. Secondary xylem, phloem, and conjunctive tissue derived as explained for fig. 4.

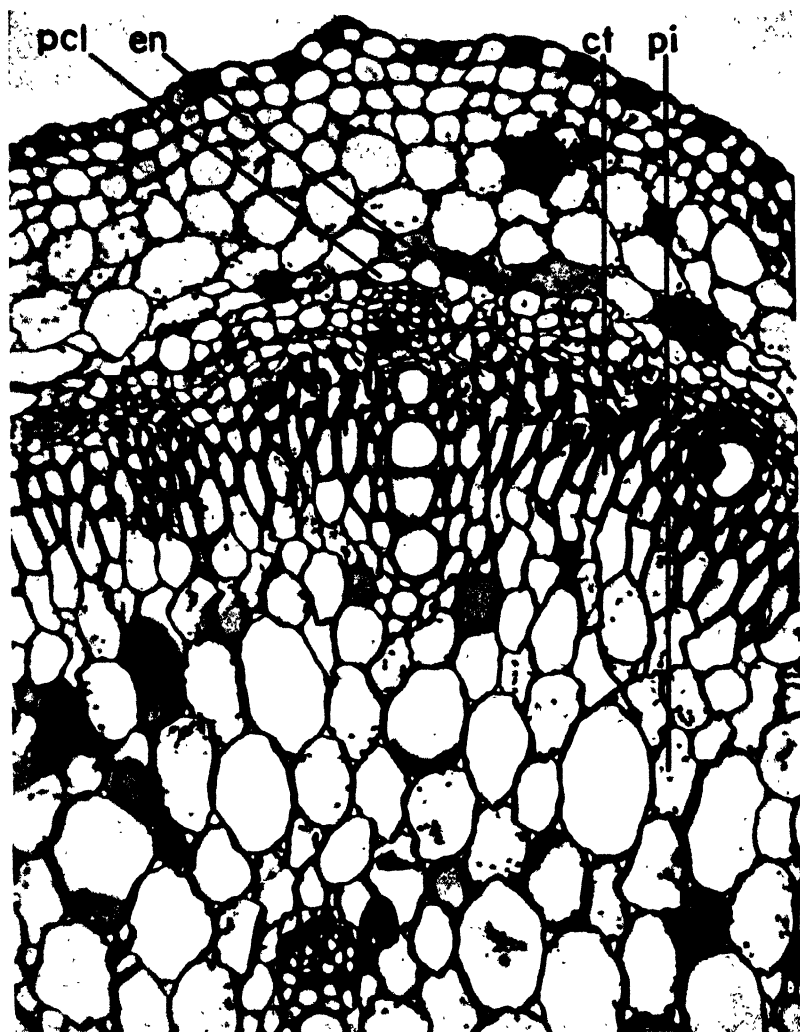


FIG. 24.—Enlarged portion as shown in fig. 23. Endodermis and pericycle not meristematic. Fascicular cambium of bundle at center continuous with interfascicular cambium derived at interfascicular parenchyma. *pcl*, pericycle; *en*, endodermis; *ct*, conjunctive tissue; *pi*, pith.

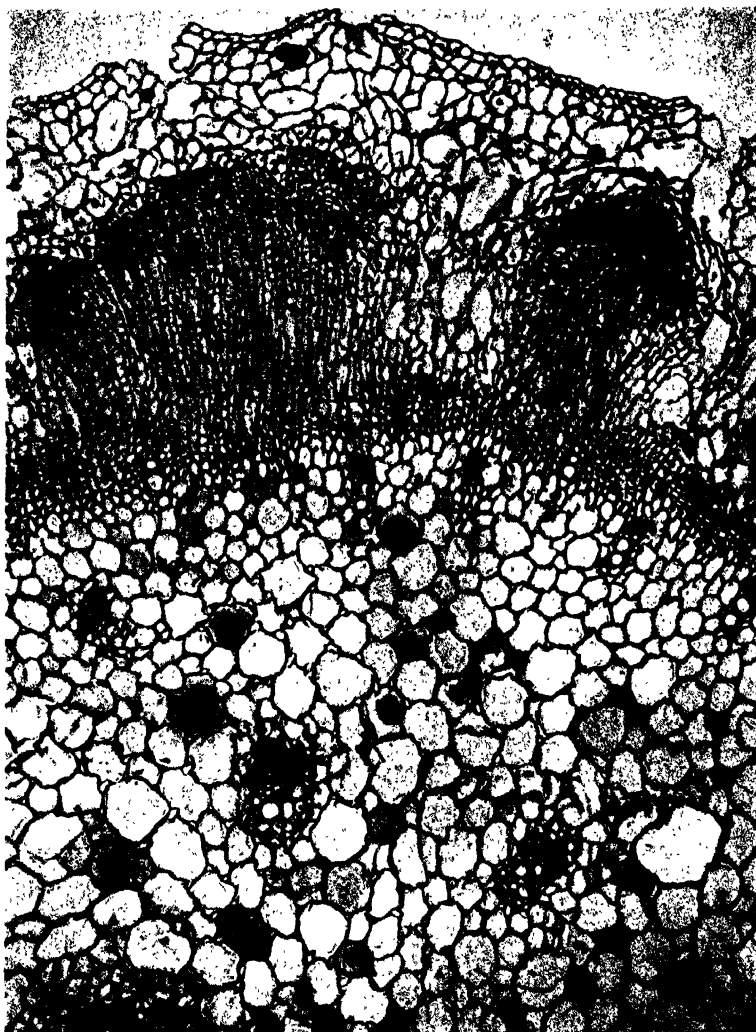


FIG. 25.—Transection of stem treated when in stage shown in figs. 23 and 24, 6 days after treatment. Tissue (except cortex and epidermis lying outside band of conjunctive tissue) shows great activity; that inside it little or no activity except for parenchymatous cells adjacent to inner vascular bundles (extreme lower left). Endodermis active.

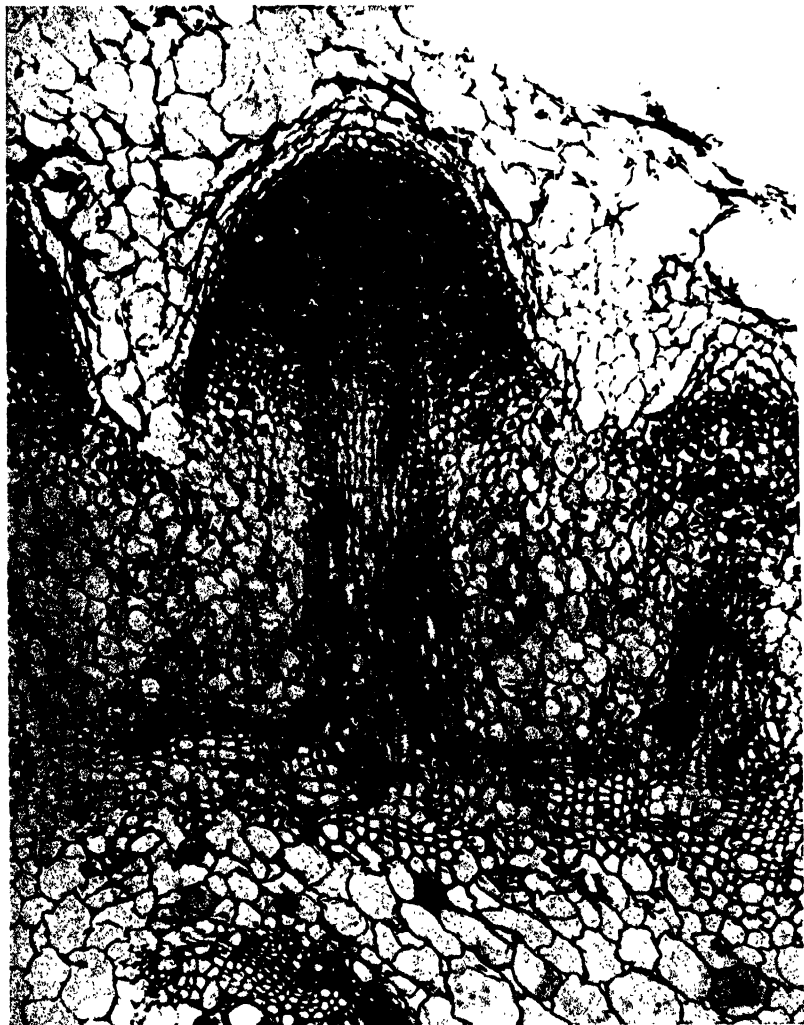


FIG. 26.—Similar to fig. 25, 11 days after treatment. Differentiation of provascular strands of root secondary to elements in band of conjunctive tissue, not to xylem nor inner vascular bundles. *Cf.* figs. 13 and 16.



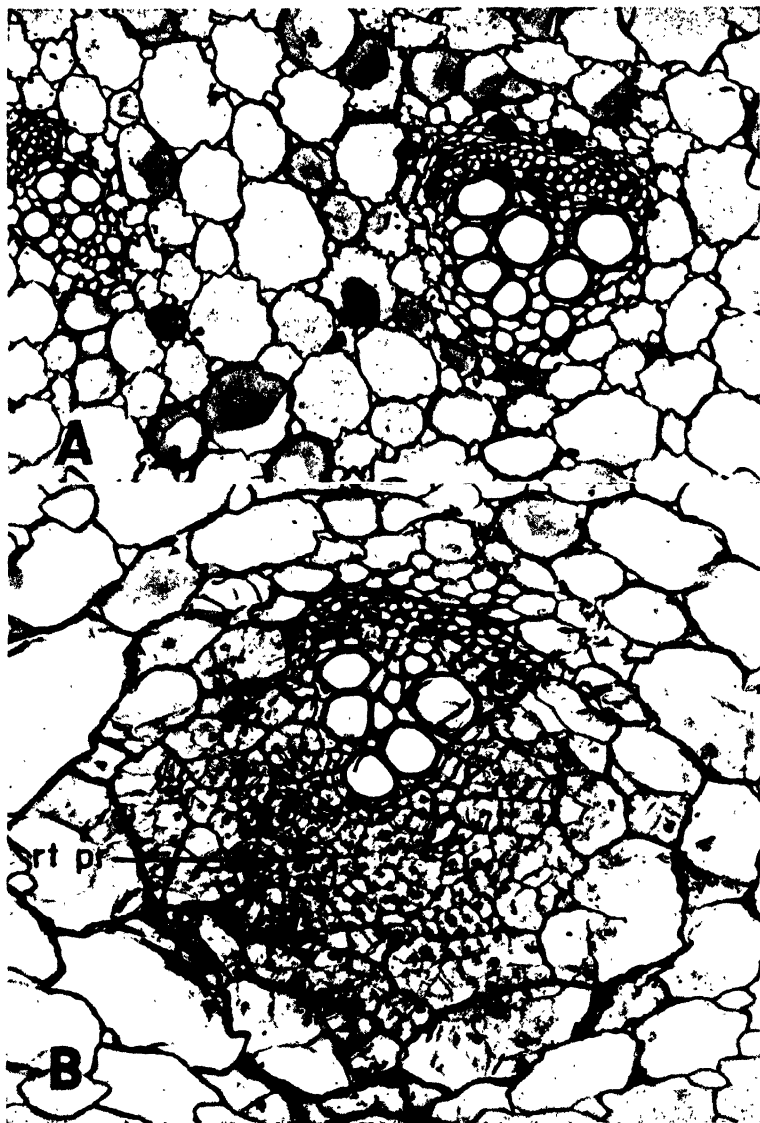


FIG. 27.—*A*: central portion of first internode of untreated plant at time fourth internode has elongated (fig. 2); pith not meristematic. Inner vascular bundle shows some secondary xylem and phloem. *B*: similar portion from plant 6 days after decapitation and treatment. Root primordium derived from proliferated parenchyma, adjacent to xylem of inner vascular bundle.

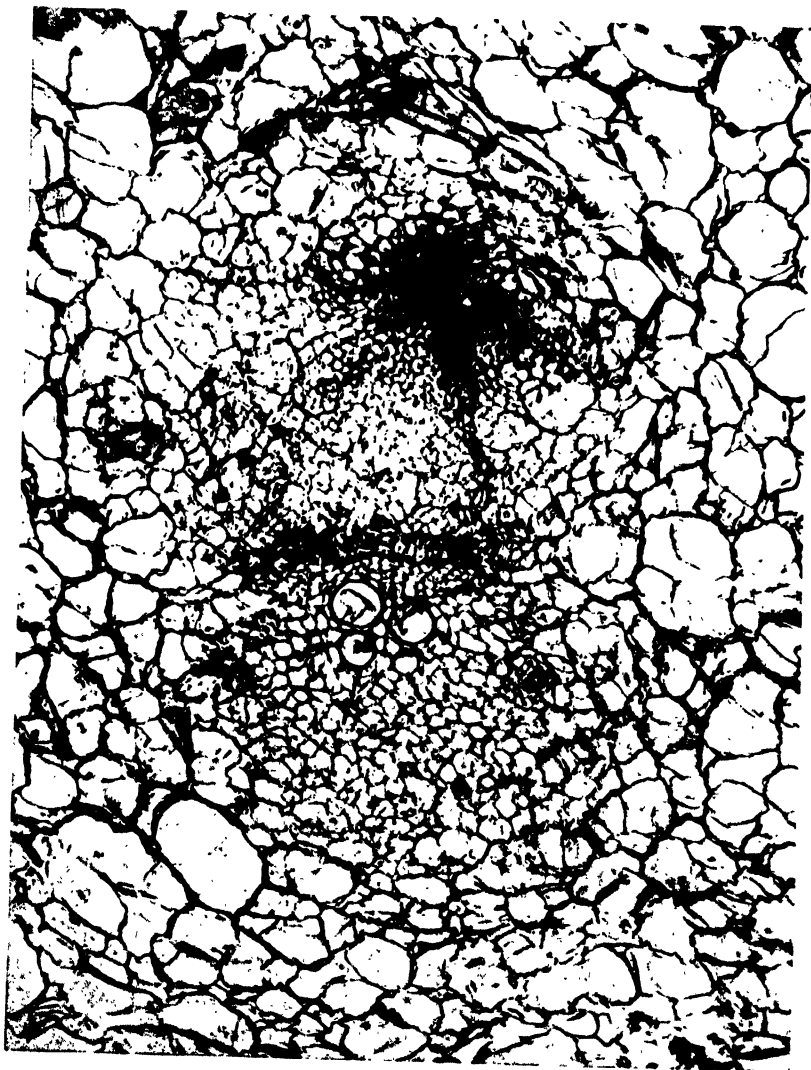


FIG. 28.—Similar to fig. 27*B*, but 11 days after treatment. Root primordium in this case derived from proliferated parenchyma over phloem of inner vascular bundle.

### Discussion

In their response to indoleacetic acid in lanolin, the tissues of the stem of *Mirabilis jalapa* resemble those of the bean in the production of large tumors from the pith which may continue growth over a long period of time, but differ sharply from the bean in the far lesser activity of the endodermis, phloem, and cambium. The derived vascular bundles in the bean originated from nearly all tissues except the epidermis and cortical parenchyma, whereas in four o'clock they are derived mainly from the pith adjacent to vascular bundles already present in the stems at time of treatment.

Certain derivatives of the pith differentiate as adventitious roots, which may be directed toward the center of the axis, as was the case in *Iresine*. The epidermis and cortical parenchyma of all four plants (bean, tomato, *Iresine*, and *Mirabilis*) respond to treatment in much the same way. In its response, the endodermis of *Mirabilis* closely resembles that of tomato. The pericycle of *Mirabilis* and *Iresine* are sensitive to treatment and respond in much the same manner, in sharp contrast to the reaction of the pericycle of bean and tomato in which the pericycle is relatively unresponsive.

The responses of the vascular tissues of *Mirabilis* differ appreciably from the responses of any of the other three plants, in that the xylem, phloem, and fascicular cambium of the inner bundles at least are much less responsive. The cambium of the small outer bundles, which is continuous with the interfascicular cambium, is the principal vascular tissue which has been observed actively to divide in response to treatment.

The production of adventitious roots in tomato, *Iresine*, and *Mirabilis* is similar with respect to development and behavior. The origin of roots was in tomato from external phloem or pith parenchyma adjacent to internal phloem; in *Iresine* from pericycle or phloem or from pith parenchyma adjacent to primary xylem; and in *Mirabilis* from pericycle, interfascicular parenchyma, and pith parenchyma adjacent to primary xylem.

The effect of the age of the stem upon its response to treatment was much the same in *Mirabilis* and *Iresine*. In both plants the older stems possess a band of conjunctive tissue which seems to in-

hibit the establishment of vascular connections between the developing roots outside the band and the vascular tissue central to it. In *Iresine* the band of conjunctive tissue was observed to undergo some response to treatment; such a response was not observed in *Mirabilis*.

The fact that decapitated first internodes if untreated with the lanolin mixture are abscised in a few days, but continue to develop for long periods of time if treated, is of special interest, both in the problems of food conduction and of tissue differentiation.

The behavior of the untreated decapitated first internodes, in contrast to those treated with the lanolin mixture, is generally in keeping with those of bean, provided the latter fails to develop a callus from the phloem. SNOW (14) has shown a direct correlation between application of growth substances and cambial activity, and MITCHELL and MARTIN (13) and MITCHELL and HAMNER (12) have shown that although the untreated, decapitated internodes of red kidney bean are not abscised, they fail to increase markedly in volume or in dry weight, but do both when treated with indoleacetic acid-lanolin mixture. In a measure the lanolin mixture may substitute for the apical meristem and other meristematic regions which had been removed above the internode, in furnishing the stimulus which results in continued activity of the secondary meristems in the internode.

Just what the nature of this stimulus may be or how its effects are brought about still remains to be determined. The conduction of foods and nutrients as well as the multiplication, enlargement, and differentiation of cells is involved. The very low concentrations of indoleacetic acid used by MITCHELL and HAMNER, which were effective in influencing the distribution of dry weight throughout the entire bean seedling, indicate that a concentration gradient of the indoleacetic acid or some derivative from it becomes established downward from the treated surface. Such a gradient is associated with a flow of food and nutrients upward in the internode, thus enabling the tissues of the internode to grow, differentiate, and mature. The influence of the treatment is not limited to the internode just below the treated surface. In bean, adventitious roots may be produced the entire length of the stem and hypocotyl, and

other histological details are readily observable in decreasing degree away from the immediate region of application (6, 8).

The question arises whether the presence of additional meristems, such as root initials, buds (2), vascular strands, and the like, which collectively are designated as the tumor, may in themselves constitute a stimulus which causes the continued flow of nutrients to them or effects the continued differentiation of tissues below them. Such is probably not the case. Histological evidence shows that there is a decreasing gradient of activity of the various tissues away from the surface of application. As a rule tumors develop following an application of lanolin mixtures having a relatively high content of growth substance (3, 6, 7, 8).

There is frequently a sharp basal limitation of tumors developed following decapitation and apical application of lanolin mixture, the tissues more distant from the treated surface responding in a manner similar to those at or near the treated surfaces when dilute mixtures are applied.

The results of MITCHELL and HAMNER on bean as well as those on *Mirabilis* demonstrate clearly that tumor formation does not necessarily follow applications of low concentrations of indoleacetic acid. With certain concentrations no tumor is formed, and the development of the tissues of the decapitated treated internodes so closely parallels that of untreated undecapitated plants that histological differences between them can scarcely be detected. Thus the presence of an induced tumor, apical or otherwise, is not necessarily a primary agent in determining the course of development of tissues at distances from it; rather this is determined by the concentration of the growth substance in contact with the cell and the particular environmental conditions such as temperature, light, and moisture prevailing during the developmental period.

### Summary

1. Seedlings of *Mirabilis jalapa* were decapitated at two different ages by severing the stem in the upper portion of the first internode, and the cut surface treated with a mixture of indoleacetic acid in lanolin. Similarly decapitated plants were treated with pure lanolin or untreated to serve as controls.

2. Gross observations and histological studies were made at regular intervals for several weeks on both the treated and control plants.

3. The first internode of decapitated plants fails to continue to enlarge if the cut surface is untreated or treated with lanolin only, and after about two weeks it dies. If the cut surface is treated with a 2 per cent lanolin-indoleacetic acid mixture, a tumor is formed near the top of the treated internode and the lower portion of the internode continues to enlarge.

4. There is wide variation in the histological responses of the various tissues. The pericycle, interfascicular parenchyma just inside it, and the interfascicular cambium (in the older stems) were the most responsive tissues. The vascular tissues were generally unresponsive. Epidermis and cortical parenchyma showed little response and the endodermis considerable. Cells of the pith were slow in initiation of the response but after activity had begun they proliferated rapidly and over a long period of time. Derivatives of the pith differentiated as internal roots or as strands of vascular tissue interspersed with parenchymatous cells. Activity in the pith was greatest adjacent to the vascular bundles.

5. Those roots which penetrated the cortex and epidermis were differentiated from derivatives of the pericycle or from tissue derived from both pericycle and interfascicular parenchyma or cambium.

6. The responses of the stems of *Mirabilis jalapa* are compared with those of red kidney bean, tomato, and *Iresine lindenii*.

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## CURRENT LITERATURE

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*Genetics and the Origin of Species.* By THEODOSIUS DOBZHANSKY. New York: Columbia University Press, 1937. Pp. 364. \$3.60.

The enthusiasm aroused by DE VRIES's presentation of his mutation theory was one of the principal causes of the rapid development of genetics in the early 1900's. It was widely believed that the study of mutation was about to lead to an immediate solution of the problems of the origin of species.

This early enthusiasm soon met disappointment. Much was indeed learned about heredity within species, and in certain special cases it became reasonably certain that recognized species owed their origin to a simple genetic mechanism, polyploidy. But it also became clear that the differences between closely related species are usually of a far higher order of complexity than the gene mutations or chromosome aberrations of the laboratory.

In recent years there has been an attempt to bridge the gap by treating the origin of species as a question of the transformation of populations rather than of individuals, to be approached by working out the statistical consequences of genetic mechanisms in populations subject to various conditions. This is essentially a return to DARWIN's viewpoint but at a higher level of definiteness.

The time has been ripe for a synthesis, bringing into proper relation to one another the great stores of information which have accumulated on mutations of all sorts, on the genetic compositions of wild populations, on species differences, and on the mechanisms of hybrid sterility. It is such a synthesis that DOBZHANSKY has attempted and, in the reviewer's opinion, achieved with eminent success.

The first chapter is introductory in character. The relations of various lines of attack on the evolutionary problem are discussed. The author definitely limits the scope of his discussion to the mechanisms of speciation, excluding all consideration of the course of phylogeny, and hence of higher categories than the species.

The second chapter deals comprehensively with gene mutation in the laboratory (types, adaptive value, multiplicity of effects, rate of occurrence under normal conditions and under special conditions, etc.). This is followed by a chapter on mutations as a basis for racial and specific differences. The author accepts the view that a race is characterized genetically by a system of gene frequencies in moving equilibrium rather than by a single typical genetic complex. The evidence for non-mendelian differentiation is discussed but the author concludes that this can hardly play more than a subordinate role. Chapter IV



gives an extensive review of types of chromosomal changes and their evolutionary significance.

After a consideration of the raw materials of evolutionary change, the statistical implications of the mendelian mechanism are introduced in chapter V. The principle of equilibrium in the absence of evolutionary pressure and the statistical effects of recurrent mutation and of inbreeding are considered and related to studies of natural populations. This is continued in the next chapter with reference to selection.

While the emphasis is on processes of gradual transformation, it is recognized that evolution is not a stereotyped process. Chapter VII deals with the evidence for abrupt speciation, as occurring rather frequently in higher plants and occasionally at least in animals, by auto- and allopolyploidy.

Chapter VIII gives a classification and discussion of the isolating mechanisms that make possible the multiplication of species. This is continued in chapter IX with respect to the problem of the nature and origin of the hybrid sterility that in most cases makes speciation an irreversible process. The contrast between sterility due to failure of pairing between differently arranged chromosomes and that due to gene effects is especially stressed. The final chapter takes an affirmative view on species as natural units, in spite of difficulties of definition.

An extraordinary amount of factual material is included but is so thoroughly digested and organized that the book is highly readable throughout. One of the most valuable features for American readers is the attention paid to European and especially Russian authors whose work has often been overlooked here. It is a book which will be a necessity for all interested in the recent development of the theory of evolution.—SEWALL WRIGHT.

*A Bibliography of Eastern Asiatic Botany.* By ELMER D. MERRILL and EGBERT H. WALKER. Jamaica Plain, Massachusetts: Arnold Arboretum of Harvard University, 1938. Pp. xlii+719. \$12.50.

This is an imposing work involving many years in its preparation, and covering "the area from western Tibet to Japan and Formosa, including China proper, Mongolia, Manchuria, Korea, and eastern and southern Siberia, and the period from the beginning of printing, not only in Europe but also in the Orient, to the end of 1936." As might be expected from a field so vast, the author-entries are numerous, totalling more than 21,000. These were obtained by the examination of over 1200 complete or partial sets of periodicals. They represent not only the various European languages but also many articles in Chinese and in Japanese. Each entry gives, following the author's name, the exact title and bibliographic citation, also a brief but usually very helpful annotation as to the nature of the cited material. The bibliography proper, covering 550 pages, is followed by "an appendix containing references to the older Chinese and Japanese botanical works, reference lists of Chinese and Japanese

botanical periodicals, and oriental authors. In the appendix Chinese and Japanese characters are used. In the bibliography proper, as well as in the appendices, the Slavic, Chinese, and Japanese titles are translated, or both transliterated and translated," a feature that greatly enhances the value of the work for most students.

The introduction states that the main objective "has been to record those papers to which botanists who are concerned with the study of plants of this area must or should refer. Papers devoted entirely to physiology, morphology, cytology, and genetics, and those without distinct taxonomic, geographic, or economic significance are omitted." The range of treatment is comprehensive, including not only the phanerogams but also the cellular and vascular cryptogams. The point of view in selection of references is said to have been chiefly taxonomic, but users will find cited a wealth of papers dealing with exploration, history of botany, bibliography, phylogeny, pathology, agriculture, horticulture, materia medica, pharmacy, biography, and divers kindred subjects. The periodical list of more than 1200 entries contains a complete reference list of serial abbreviations, with the respective fully expanded title for each, and pertinent cross references and comment. Especially noteworthy is the series of comprehensive and detailed general, regional, and systematic indices provided near the end of the volume. These cover more than 125 pages, and make the vast array of assembled data much more useful because much more usable.

In the field of bibliographic botany, this work will long stand as a monumental contribution, as well as an eloquent tribute to the untiring zeal and astute scholarship of its two authors.—E. E. SHERFF.

*Plant Ecology.* By JOHN E. WEAVER and FREDERIC E. CLEMENTS. 2d ed. New York: McGraw-Hill, 1938. Pp. xxii+601. Figs. 271. \$5.00.

The purpose of this revised edition is "to furnish a comprehensive textbook in accord with present-day ecological progress and a guide to workers in the numerous related fields where an intimate knowledge of plants and plant environments, whether natural or modified by man, is fundamental to progress."

The book has been enlarged by about eighty pages. About sixty new illustrations are included, and fifty of the old ones have been dropped. More than four hundred titles have been added to the bibliography.

This volume differs from the original in a number of ways. The number of chapters remains the same, but their position has been altered somewhat. The chapter on Plant Succession has been placed ahead of the discussion of the Units of Vegetation, thus forming a background for CLEMENTS' system of nomenclature of climax and seral units.

Important revisions and additions have been made in and to the chapter on Methods of Studying Vegetation, following developments in America of the last decade. The methods of the plant sociologists are given less than a page, and are dismissed as of little or insufficient value. In the chapter on Succession,

CLEMENTS' current ideas of subclimax, preclimax, postclimax, and disclimax are elucidated at length, in addition to the old material. The climax units are discussed first in the chapter on Units of Vegetation followed by the seral ones. Of CLEMENTS' recently proposed terms, only faciation and facies have been added to this chapter.

Parts of the chapter on soils have been altered considerably, and a much more comprehensive picture of soil texture and structure, of the processes of soil development, and of the major soil groups is given, interpreted in accord with modern soil science. The chapter on Reaction and Stabilization has been slightly lengthened and drastically changed, the emphasis being placed upon the reactions of vegetation of importance in conservation. This leads up to the chapter on Coaction and Conservation, which includes the more or less unrelated topics of pollination, and conservation of soil, water, and wild life. The discussions of light and adaptation to water have been modernized to a great extent, especially the treatment of xerophytes. Some authorities, however, may still find reasons for criticism.

The most interesting changes in the chapters on Plants and Plant Communities as Indicators, and Climax Formations of North America, have to do with CLEMENTS' efforts to fit his more recent terms and concepts to the climax units previously recognized. Of these, the inclusion of most of Illinois, Missouri, and Iowa in the "true prairie" climax grassland will attract most attention.

In spite of certain deficiencies and opinions which conflict with the ideas of other authorities, this book will continue to be the most valuable one-volume general text and reference work in plant ecology in use in this country.—C. E. OLMSTED.

*An Ecological Glossary.* By J. RICHARD CARPENTER. Norman, Oklahoma: University of Oklahoma Press, 1938. Pp. x+306. Appendix of maps and tables. \$4.00.

In the words of the compiler of this glossary, his purpose, a commendable one, has been "to bring together and make available the more technical and restricted usages of terms which have been and are in the ecological literature. While no pretense is made that the list is by any means exhaustive, nearly all of the technical terms encountered in a diligent search of the current periodicals and texts are included." Examined according to this purpose, one finds that about 3000 terms are defined. A large number of the definitions are copied directly without editing or amplifying from CLEMENTS' publications of 1902, 1904, and 1905, and from JACKSON's Glossary of Botanic Terms. Many obsolete terms used only in the publications in which they were proposed are thus included, while modern terms and modern usages are often lacking. In addition to the errors of inclusion and omission, there are numerous incorrect citations and inaccurate definitions, errors in spelling, both typographical and otherwise, and frequent failure of the cross reference system. In the opinion of the reviewer,

these marks of hasty preparation and lack of balance more than offset the real value of the book, and may hinder, rather than aid, the stabilization of ecological nomenclature and the advance of the science.—C. E. OLMSTED.

*The Structure and Development of the Fungi.* By H. C. I. GWYNNE-VAUGHAN and B. BARNES. 2d ed. Cambridge: University Press. New York: Macmillan Co., 1937. Pp. 449. Illustrated. \$5.50.

The second edition of this well known textbook has been entirely reset and revised to include the advances in the knowledge of the fungi during the last decade. The new book contains sixty-five additional pages of text material and twenty-four new figures.

The organization remains essentially unchanged. The introduction deals with the general characters of the fungi. Several new topics and much other new material have been included in the various chapters; as, for example, the significance of flagellation as a guide to the interrelationships of the Phycomycetes, heterothallism in the rusts, and a number of new life histories. Some forty pages are devoted to the literature, covering approximately 1000 titles. The final chapter, which deals with mycological technique, should prove useful to students.

The book is characterized by its conservatism in classification and treatment. The authors apparently have been hesitant to accept some of the more significant ideas of GAUMANN: for example, the importance of the degree of development of the dicaryophase as a basis of the systematic classification of the Ascomycetes and the structure and course of development of the basidia in the Basidiomycetes. They also have failed to include some of the newer research reported in the literature of recent years. One cannot find, for instance, HANNA'S work on *Ustilago zeae*, in which he reports invasion of the host by haplophase mycelia formed by germination of the sprout cells. On the whole, however, the book is carefully and critically done, and it will continue to be a valuable text for students interested in the morphology of the fungi.—J. M. BEAL.

*Catalogue of the Flora of the State of Texas.* By V. L. CORY. Texas Agr. Exp. Station: Bull. 550, 1937. Pp. 130.

*Valuable Plants Native to Texas.* By H. B. PARKS. Texas Agr. Exp. Station: Bull. 551, 1937. Pp. 173.

The recent catalogue of the vascular plants of Texas, prepared by CORY, is the first attempt to unify the diversified and scattered taxonomic literature of this floristically important state. Technical and common names follow the International Rules of Botanical Nomenclature and the Standardized Plant Names, respectively. By means of key numbers which follow each plant name, the distribution of that species may be ascertained by consulting the simplified

map of the state. In all 162 families, 1063 genera, and 5099 species and varieties are listed. The nomenclature is in accordance with the newest researches, and many of the older, now invalid, names are given parenthetically.

PARKS has discussed the economically important plants of Texas in a recent state publication. Arranged by families in the conventional manner, nearly 1000 native plants are listed and described for their success or possible success in ornamental plantings, in erosion control, in shading, or for any other useful purpose. A very complete index is an aid to amateurs and professional botanists alike.—P. D. VOTH.

*Determination of the Amino Acids.* By RICHARD J. BLOCK. Minneapolis: Burgess Publishing Co., 1938. Pp. iii+85. Illustrated. \$2.00.

The literature dealing with the quantitative and semiquantitative determination of the individual amino acids is very widely scattered. The methods now in use for such purposes have been compiled in this volume, which will serve a useful purpose wherever amino acids are to be determined. Unfortunately plant physiologists are not yet in a position to interpret the functions of the individual amino acids in growth processes, and determination of the mixtures found in protein hydrolysates is hardly useful; nevertheless there will be constantly increasing attention given to the nature of the nitrogen fractions now recognized as important in the physiological behavior of the organism, in proportion to the sharpening of our tools for such investigations.

There are eleven chapters, the first five of which consider related groups of amino acids together. These groups are as follows: Arginine, histidine, and lysine; tyrosine, tryptophane, dihydroxyphenylalanine, and thyroxine; proline and hydroxyproline; cystine, cysteine, and methionine; and glutamic acid, aspartic acid, and hydroxyglutamic acid.

The next five chapters cover the determination of amino acids which do not fall into group classifications. These are alanine, glycine, leucine, phenylalanine, and serine. The final chapter brings together various methods for the separation of amino acids in mixtures. Included are the methods of FISCHER, CHERBULIEZ, DAKIN, BRAZIER, and PRZYLECKI and KASPRZYK.

The apparatus diagrams are very useful. Each chapter is supplied with a bibliography. While the work is obviously a compilation, it will be appreciated for the time it will save in looking up methods; adequate critical information as to the limitations and the precautions to be employed will have to be sought in the original papers.—C. A. SHULL.

*Die Pilze Mitteleuropas.* Editorship H. Kniep, P. Claussen, and J. Barz. Werner Klinghardt, 1935.

Since the last mention in this journal (97:686. 1936) of *Die Pilze Mitteleuropas*, there have been issued numbers 17 and 18 of volume I (Boletaceae by

KALLENBACH), and numbers 4, 5, and 6 of volume II (Tremellineae by NEUHOF and Lactarii by KNAUTH and NEUHOF). These numbers are characterized by the same excellency as the previous ones.—G. K. K. LINK.

*Die mikroskopischen Boden Pilze.* By ANNELIESE NIETHAMMER. The Hague: W. Junk, 1937. Pp. 193. Illustrated.

The author of this treatise on soil fungi states that this "pioneer work" is intended to create a considerable number of workers devoted to the field of microscopic fungi of soils. The material is sketchily presented under the following chapter headings: Systematic survey of fungus forms; Distribution areas; Fungus cycles in nature; Pathology; Functions; Growth regulators; Fertilizer problems. The most significant contribution is the second chapter. The publication should be of interest and profit to specialists in soil problems.—G. K. K. LINK.



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